BIOMÉRIEUX

# BACT/ALERT® FAN® PLUS

**BLOOD CULTURE MEDIA**

## The fastest patient results. Delivered.


PIONEERING DIAGNOSTICS

# BACT/ALERT® FAN® PLUS blood culture media

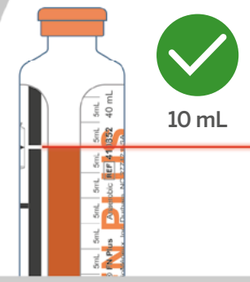
## HOW CAN YOU HELP PUT TIME ON THE PATIENT'S SIDE?

### WHY CHOOSE BETWEEN FAST AND ACCURATE?

**Innovative polymeric beads offer both rapid results and superior accuracy compared to resin technology.**


### WHY CHOOSE BETWEEN EASY ROUTINE AND OPTIMAL PERFORMANCE?

**Make your routine testing easier with smart, reliable blood culture media bottles.**




## RAPIDITY

- Shorter time to detection through earlier organism recovery [1–3]
- Faster antibiotic neutralization [1]
- Even faster when used with BACT/ALERT® VIRTUO® [4]

## RELIABILITY

- Enhanced binding kinetics and antibiotic neutralization improve overall microorganism recovery [1]
- Recovery for wide range of organism/drug combinations including:
  - Levofloxacin / *K. pneumoniae*
  - Vancomycin / *S. aureus*
  - Amikacin / *E. coli*

## EFFICIENCY

- Only 2 bottle types to manage* to detect a wide range of bacteria and even yeast [5,6]
- FAN® PLUS bottles** cleared for blood and sterile body fluid samples
- Improved Gram stain reading for rapid, smooth transition to downstream tests
- Safe, easy-to-manage plastic bottles

## INGENUITY

- Visual colorimetric change enables immediate action on delayed-entry positive bottles
- Fill-to mark guides optimum blood volume collection for improved result reliability [6]
- Systematic volume control lets you act immediately if needed

* 2 bottles for adult patients for optimal recovery of potential pathogens; a third bottle type is available for pediatric patients and is only intended for detection form blood.

** PF Plus - 410853
FN Plus - 410852

# TIME IS OF THE ESSENCE IN SEPSIS DIAGNOSTIC MANAGEMENT

**SAMPLE COLLECTION & TRANSPORTATION**
**reduced by 7 hours[7]**

FAN® PLUS MEDIA & WORKFLOW

**BLOOD CULTURE**
**reduced by 3.5 hours[8]**

BACT/ALERT® VIRTUO®

**PATHOGEN IDENTIFICATION**
**reduced by 23 hours[9]**

FILMARRAY®

**reduced by 5 hours[9]**

VITEK®MS

**ANTIBIOTIC SUSCEPTIBILITY TESTING**
**reduced by 1 day[10]**

VITEK®2

**SEPSIS SUSPICION: VIDAS® B·R·A·H·M·S PCT™**

**ANTIBIOTIC MANAGEMENT: VIDAS® B·R·A·H·M·S PCT™**

Innovation in microbiology must never stop – because your laboratory challenges never stop. For more than 50 years, bioMérieux has shared your commitment to continually strengthen laboratory impact on patient therapy.

BACT/ALERT® FAN® PLUS is part of our integrated blood culture offer. Together with our ID/AST* offer, they meet your needs from the most routine to the truly challenging.

Our offers let you leverage your expertise to deliver test results that impact timely, appropriate therapy.

* ID/AST: identification and antimicrobial susceptibility testing.



REFERENCES

1- Lovern, D. *et al*. "Antimicrobial binding and growth kinetics in BacT/ALERT FA Plus and BACTEC Aerobic/F Plus blood culture bottles." Eur J Clin Microbiol Infect Dis., Dec. 2016.
2- Amarsy-Guerle, R. et al. High medical impact of implementing the new polymeric bead-based BacT/ALERT® FAPlus and FNPlus blood culture bottles in standard care. Eur. J. Clin. Microbiol. Infect. Dis. 34, 1031–1037 (2015).
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4- Cheong Y.S., Chew K.L., Jureen R. «Evaluation of the BacT/ALERT VIRTUO and the BacT/ALERT 3D automated microbial detection systems», ECCMID 2016 – Poster P0960
5- McDonald, C. *et al*. "Controlled Comparison of BacT/ALERT FAN Aerobic Medium and BACTEC Fungal Blood Culture Medium for Detection of Fungemia." JCM, Feb., 2001.
6- Bourbeau, P.P., Pohlman, J.K. "Three Days of Incubation May Be Sufficient for Routine Blood Cultures with BacT/Alert FAN Blood Culture Bottles." JCM, Jun., 2001.
7- Internal data – USA performance Solution Dept, based on 450+ customer assessment where the average time to arrival to the lab of an inoculated blood culture bottle has been equal to 10 hours, and has been reduced on average by 70%
8- Deol P. *et al*., BacT/ALERT® VIRTUO®: rapid time to detection difference between the BacT/ALERT® VIRTUO® and the BacT/ALERT® 3D®", ECCMID 2016, Poster #EV0459
9- Potential pathogen ID time reduction compared to VITEK 2, refer to Instruction For Use (non-binding data)
10- Römmler W., *et al*. ASM 2006; Poster C-123.



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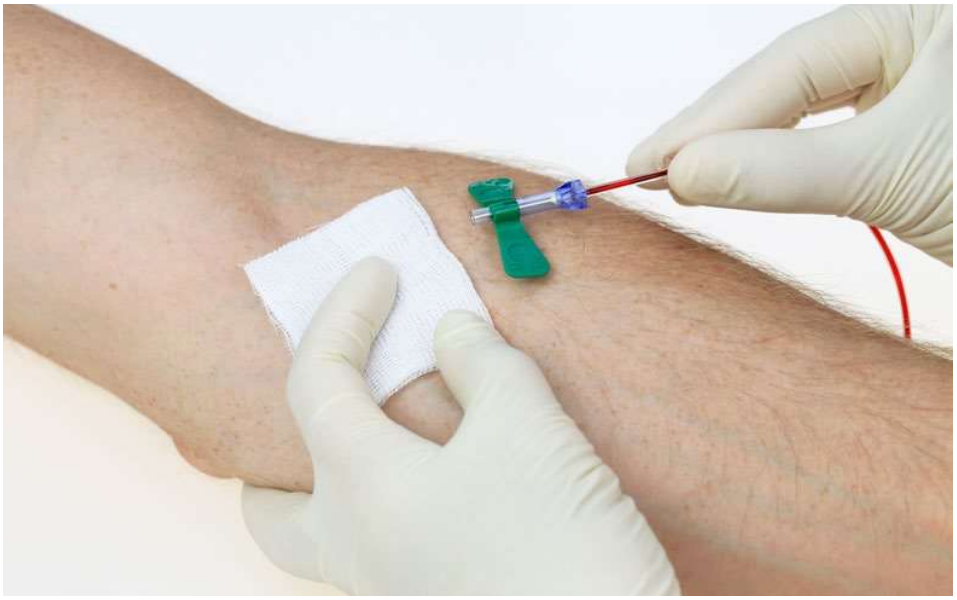


/ You can also find out more about our safety products at www.power4safety.com.

# SAFETY BLOOD COLLECTION SET

**The SAFETY Blood Collection Set was developed especially for patients with difficult vein conditions, but is also suitable for any other blood collection situation, as it offers the patient a particularly gentle collection comfort.**

The product offers a high level of safety by manually activating the protective mechanism while the needle is still inside the vein. The correct activation by the user is indicated by a clearly audible click. The safety blood collection set thus provides the best possible protection against needlestick injuries. The visual control of the puncture through a transparent viewing window also increases the puncture safety. The product can be used for blood collection or for infusions for up to five hours.

The SAFETY Blood Collection Set is a sterile single use winged needle set connected to a flexible tube, with or without a Luer adapter or Luer adapter with holder. The product is ergonomically and individually packed, making it easy to open and causing little waste.

- A viewing window between the needle and the tube shows whether the venipuncture was successful
- Tube with different lengths offers more flexibility in blood collection
- Simple activation of the safety mechanism

Technines specifikacijos reikalavimai:

2.4.1, 2.4.2, 2.5.2, 2.6.2


## SAFETY Blood Collection/Infusion Set

This version of the product can be used for blood collection by assembling it with, for example, a HOLDEX® or VACUETTE® SAFELINK, or for short-term infusion for up to 5 hours instead.

*Safety mechanism: manual activation*

| Item no. | Colour code | Needle size | Needle length | Needle wall type | Tubing length | Tubing length | Qty. inner / outer |
|---|---|---|---|---|---|---|---|
| 450091 | green | 21 G | 19 mm | thin wall | 7.5″ | 19 cm | 50 / 1,000 |
| 450191 | green | 21 G | 19 mm | thin wall | 12″ | 30 cm | 50 / 1,000 |
| 450092 | blue | 23 G | 19 mm | thin wall | 7.5″ | 19 cm | 50 / 1,000 |
| 450090 | orange | 25 G | 19 mm | thin wall | 7.5″ | 19 cm | 50 / 1,000 |


## SAFETY Blood Collection Set + Luer Adapter

This version of the SBC set has a Luer adapter and can be used with a holder such as our standard tube holder or blood culture holder.

*Safety mechanism: manual activation*

| Item no. | Colour code | Needle size | Needle length | Needle wall type | Tubing length | Tubing length | Qty. inner / outer |
|---|---|---|---|---|---|---|---|
| 450083 | green | 21 G | 19 mm | thin wall | 4″ | 10 cm | 50 / 1,000 |
| 450081 | green | 21 G | 19 mm | thin wall | 7.5″ | 19 cm | 50 / 1,000 |
| 450095 | green | 21 G | 19 mm | thin wall | 12″ | 30 cm | 50 / 1,000 |
| 450084 | blue | 23 G | 19 mm | thin wall | 4″ | 10 cm | 50 / 1,000 |
| 450082 | blue | 23 G | 19 mm | thin wall | 7.5″ | 19 cm | 50 / 1,000 |
| 450096 | blue | 23 G | 19 mm | thin wall | 12″ | 30 cm | 50 / 1,000 |
| 450099 | orange | 25 G | 19 mm | thin wall | 12″ | 30 cm | 50 / 1,000 |


## SAFETY Blood Collection Set + Holder

This version of the SBC set is manufactured with a pre-assembled tube holder and therefore allows an immediate start of the blood collection without assembling with further components.

*Safety mechanism: manual activation*

| Item no. | Colour code | Needle size | Needle length | Needle wall type | Tubing length | Tubing length | Qty. inner / outer |
|---|---|---|---|---|---|---|---|
| 450087 | green | 21 G | 19 mm | thin wall | 4″ | 10 cm | 24 / 240 |
| 450085 | green | 21 G | 19 mm | thin wall | 7.5″ | 19 cm | 24 / 240 |
| 450160 | green | 21 G | 19 mm | thin wall | 12″ | 30 cm | 24 / 240 |
| 450088 | blue | 23 G | 19 mm | thin wall | 4″ | 10 cm | 24 / 240 |
| 450086 | blue | 23 G | 19 mm | thin wall | 7.5″ | 19 cm | 24 / 240 |
| 450161 | blue | 23 G | 19 mm | thin wall | 12″ | 30 cm | 24 / 240 |


## SAFETY Blood Collection Set + Blood Culture Holder

This version of the SBC set is manufactured with a pre-assembled blood culture holder. The product is suitable for blood collection with common blood culture bottles and subsequently with VACUETTE® Blood Collection Tubes if required.

Technines specifikacijos reikalavimai:
2.4.1, 2.4.2, 2.5.1, 2.5.3, 2.5.4, 2.6.1, 2.6.3, 2.6.4

*Safety mechanism: manual activation*

| Item no. | Colour code | Needle size | Needle length | Needle wall type | Tubing length | Tubing length | Qty. inner / outer |
|---|---|---|---|---|---|---|---|
| 450182 | green | 21 G | 19 mm | thin wall | 7.5″ | 19 cm | 24 / 240 |
| 450183 | blue | 23 G | 19 mm | thin wall | 7.5″ | 19 cm | 24 / 240 |
| 450184 | green | 21 G | 19 mm | thin wall | 12″ | 30 cm | 24 / 240 |
| 450185 | blue | 23 G | 19 mm | thin wall | 12″ | 30 cm | 24 / 240 |

Your Power for Health

greiner bio-one

# VACUETTE®

## SAFETY Blood Collection Sets

Our Innovations
for Your Safety

www.gbo.com/preanalytics

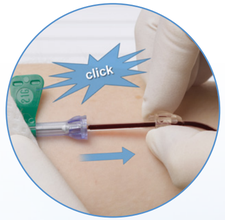
# SAFETY Blood Collection Sets

## Specially for difficult vein conditions

The SAFETY Blood Collection Set has been specially developed for patients with difficult vein conditions , but is equally suitable for any regular blood taking situation, due to the gentle comfort it provides to a patient.

As the protection mechanism is activated whilst the needle is still in the vein, a high level of safety is ensured. Correct activation via retraction is indicated by an acoustic signal.

Protect yourself and your employees from the risk of needlestick injury. Every needlestick injury is one too many.




### Transparent View Window

The transparent view window provides clear vein entry indication.

### Safety Mechanism

Simple activation of the safety mechanism via lightly pressing in both sides of the hub. Correct activation is indicated by an acoustic signal.

### Optional

It can be used for blood collection or infusion for up to five hours.

### More Flexibility

Assorted tube lengths offer more flexibility for blood collection.



# SAFETY Blood Collection Sets Combination Products

As a practical combination product, the SAFETY Blood Collection Set is also available pre-assembled.

### SAFETY Blood Collection Set + Luer Adapter

- Pre-attached set with Luer adapter in sterile blister pack.
- Handling is optimised.

Item No. 450081-84
Item No. 450095-96
Item No. 450099

### SAFETY Blood Collection Set + Holder

- Pre-attached set with holder in sterile blister pack.
- No threading necessary.
- A practical, ready-to-use solution that saves time.

Item No. 450080
Item No. 450085-88
Item No. 450160-61

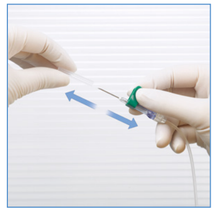
### SAFETY Blood Collection Set + Blood Culture Holder

- Pre-attached set with Blood Culture Holder in sterile blister pack.
- Designed for use with most commonly used blood culture bottles and **VACUETTE**® blood collection tubes in one procedure.
- The set is sterile and pre-assembled for convenience, cost effectiveness and ensures your patient only has to undergo one venipuncture for all procedures.

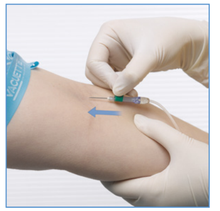
Item No. 450182-85



# Instructions for Use *



**1** Remove protective cap

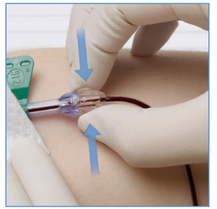


**2** Venipuncture

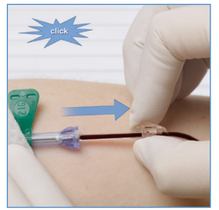
* For the complete handling recommendation please refer to the Instructions for Use for each product.

**3** Insert the tube

**4** Remove the last tube



**5** Activate the safety mechanism (by pressing down and pulling back the lighter coloured area)




**6** Audible click indicates activation

**7** Activated SAFETY Blood Collection Set


**8** Disposal

# SAFETY Blood Collection Sets

| Item. No. | Description | Size | | Packaging Inner | Outer |
|---|---|---|---|---|---|
| **SAFETY Blood Collection/Infusion Sets (sterile)** | | | | | |
| 450090 | SAFETY Blood Collection/Infusion Set | tubing length: 19cm | 25G x 3/4“ | 50 pcs. | 1000 pcs. |
| 450091 | SAFETY Blood Collection/Infusion Set | tubing length: 19cm | 21G x 3/4“ | 50 pcs. | 1000 pcs. |
| 450191 | SAFETY Blood Collection/Infusion Set | tubing length: 30cm | 21G x 3/4“ | 50 pcs. | 1000 pcs. |
| 450092 | SAFETY Blood Collection/Infusion Set | tubing length: 19cm | 23G x 3/4“ | 50 pcs. | 1000 pcs. |
| 450192 | SAFETY Blood Collection/Infusion Set | tubing length: 30cm | 23G x 3/4“ | 50 pcs. | 1000 pcs. |
| **SAFETY Blood Collection Sets + Luer Adapters (sterile)** | | | | | |
| 450083 | SAFETY Blood Collection Set + Luer Adapter | tubing length: 10cm | 21G x 3/4“ | 50 pcs. | 1000 pcs. |
| 450081 | SAFETY Blood Collection Set + Luer Adapter | tubing length: 19cm | 21G x 3/4“ | 50 pcs. | 1000 pcs. |
| 450095 | SAFETY Blood Collection Set + Luer Adapter | tubing length: 30cm | 21G x 3/4“ | 50 pcs. | 1000 pcs. |
| 450084 | SAFETY Blood Collection Set + Luer Adapter | tubing length: 10cm | 23G x 3/4“ | 50 pcs. | 1000 pcs. |
| 450082 | SAFETY Blood Collection Set + Luer Adapter | tubing length: 19cm | 23G x 3/4“ | 50 pcs. | 1000 pcs. |
| 450096 | SAFETY Blood Collection Set + Luer Adapter | tubing length: 30cm | 23G x 3/4“ | 50 pcs. | 1000 pcs. |
| 450099 | SAFETY Blood Collection Set + Luer Adapter | tubing length: 30cm | 25G x 3/4“ | 50 pcs. | 1000 pcs. |
| **SAFETY Blood Collection Sets + Holders (sterile)** | | | | | |
| 450087 | SAFETY Blood Collection Set + Holder | tubing length: 10cm | 21G x 3/4“ | 24 pcs. | 240 pcs. |
| 450085 | SAFETY Blood Collection Set + Holder | tubing length: 19cm | 21G x 3/4“ | 24 pcs. | 240 pcs. |
| 450160 | SAFETY Blood Collection Set + Holder | tubing length: 30cm | 21G x 3/4“ | 24 pcs. | 240 pcs. |
| 450088 | SAFETY Blood Collection Set + Holder | tubing length: 10cm | 23G x 3/4“ | 24 pcs. | 240 pcs. |
| 450086 | SAFETY Blood Collection Set + Holder | tubing length: 19cm | 23G x 3/4“ | 24 pcs. | 240 pcs. |
| 450161 | SAFETY Blood Collection Set + Holder | tubing length: 30cm | 23G x 3/4“ | 24 pcs. | 240 pcs. |
| 450080 | SAFETY Blood Collection Set + Holder | tubing length: 30cm | 25G x 3/4“ | 24 pcs. | 240 pcs. |
| **SAFETY Blood Collection Sets + Blood Culture Holders (sterile)** | | | | | |
| 450182 | SAFETY Blood Collection Set + Blood Culture Holder | tubing length: 19cm | 21G x 3/4“ | 24 pcs. | 240 pcs. |
| 450183 | SAFETY Blood Collection Set + Blood Culture Holder | tubing length: 19cm | 23G x 3/4“ | 24 pcs. | 240 pcs. |
| 450184 | SAFETY Blood Collection Set + Blood Culture Holder | tubing length: 30cm | 21G x 3/4“ | 24 pcs. | 240 pcs. |
| 450185 | SAFETY Blood Collection Set + Blood Culture Holder | tubing length: 30cm | 23G x 3/4“ | 24 pcs. | 240 pcs. |

These products are not made with natural rubber latex or DEHP.

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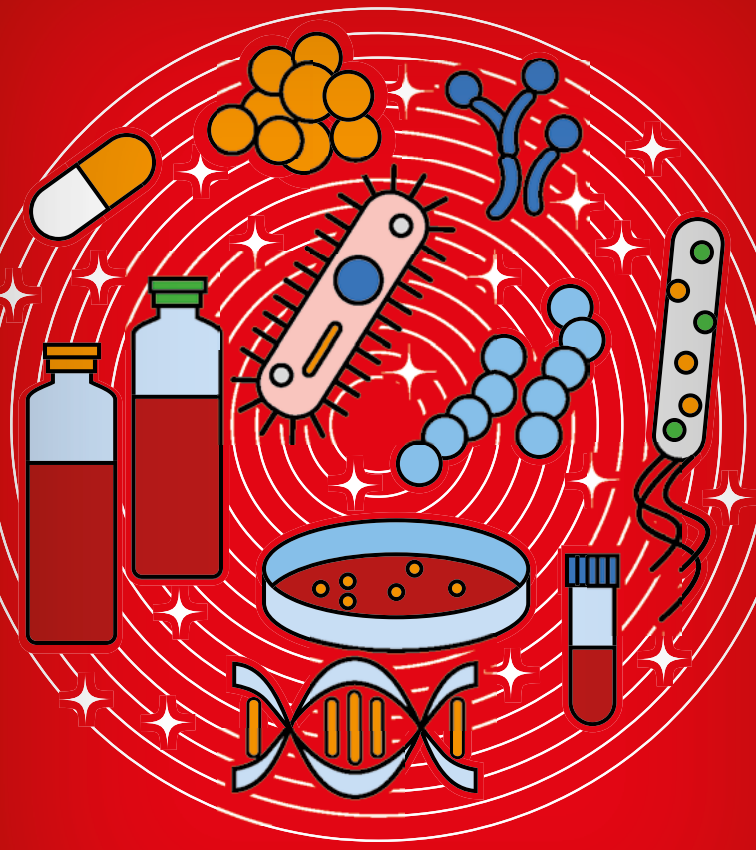
BIOMÉRIEUX

# BLOOD CULTURE

## A key investigation for diagnosis of bloodstream infections



PIONEERING DIAGNOSTICS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*for their helpful advice and comprehensive review of this booklet.*


# INTRODUCTION

"...the laboratory detection of bacteremia and fungemia remains one of the most important functions of clinical microbiology laboratories... A positive blood culture establishes or confirms that there is an infectious etiology of the patient's illness. Moreover, it provides the etiologic agent and allows antibiotic susceptibility testing for optimization of therapy." [1]

**The laboratory detection of bacteremia and fungemia** using blood cultures is one of the most simple and commonly used investigations to establish the etiology of bloodstream infections.

**Rapid, accurate identification** of the bacteria or fungi causing bloodstream infections provides vital clinical information required to diagnose and treat sepsis.

**Sepsis** is a complex inflammatory process that is largely under-recognized as a major cause of morbidity and mortality worldwide. There are an estimated 19 million cases worldwide each year,[2] meaning that sepsis causes 1 death every 3-4 seconds.[3]

**Early diagnosis and appropriate treatment** make a critical difference when it comes to improving sepsis patient outcomes. Chances of survival go down drastically the longer initiation of treatment is delayed. If a patient receives antimicrobial therapy within the first hour of diagnosis, chances of survival are close to 80%; this is reduced by 7.6% for every hour after. Yet, if a patient initially receives inappropriate antimicrobial treatment, they are five times less likely to survive.[4]

This booklet aims to:

- **answer key questions** commonly asked in relation to blood culture
- **provide practical recommendations** for routine blood culture procedures
- **offer an illustrated step-by-step guide** to best blood culture collection practices.

This booklet is intended to be a useful reference tool for physicians, nurses, phlebotomists, laboratory personnel and all other healthcare professionals involved in the blood culture process.

# DEFINITIONS

**Bacteremia:** the presence of bacteria in the blood. It may be transient, intermittent or continuous.

**Blood culture:** blood specimen submitted for culture of microorganisms. It enables the recovery of potential pathogens from patients suspected of having bacteremia or fungemia.

**Blood culture series:** a group of temporally related blood cultures that are collected to determine whether a patient has bacteremia or fungemia.

**Blood culture set:** the combination of blood culture bottles (one aerobic and one anaerobic) into which a single blood collection is inoculated.

**Bloodstream Infection (BSI):** an infection associated with bacteremia or fungemia.

**Contaminant:** a microorganism isolated from a blood culture that was introduced during specimen collection or processing and is not considered responsible for BSI (i.e. the isolates were not present in the patient's blood when the blood was sampled for culture).

**Contamination:** presence of microorganisms in the bottle that entered during sampling but were not actually circulating in the patient's bloodstream.

**Fungemia:** the presence of fungi in the blood.

**Sepsis:** life-threatening organ dysfunction caused by a dysregulated host response to infection.[5]

**Septicemia:** clinical syndrome characterized by fever, chills, malaise, tachycardia, etc. when circulating bacteria multiply at a rate that exceeds removal by phagocytosis.[6]

**Septic episode:** an episode of sepsis or septic shock for which a blood culture or blood culture series is drawn.

**Septic shock:** a subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to substantially increase mortality.[5]

*Source: Wayne, P.A. Principles and procedures for Blood Cultures; Approved Guideline, CLSI document M47-A. Clinical and Laboratory Standards Institute (CLSI); 2007 unless otherwise specified.*



# TABLE OF CONTENTS

# 1 BLOOD CULTURE ESSENTIALS

## 1 What is a blood culture?

A blood culture is a laboratory test in which blood, taken from the patient, is inoculated into bottles containing culture media to determine whether infection-causing microorganisms (bacteria or fungi) are present in the patient's bloodstream.

### → Blood cultures are intended to:

- Confirm the presence of microorganisms in the bloodstream
- Identify the microbial etiology of the bloodstream infection
- Help determine the source of infection (e.g. endocarditis)
- Provide an organism for susceptibility testing and optimization of antimicrobial therapy

**3 MAIN AIMS OF BLOOD CULTURE*:**

- **Confirm infectious etiology**
- **Identify the etiological agent**
- **Guide antimicrobial therapy**

* Adapted from ESCMID (European Society of Clinical Microbiology and Infectious Diseases) guidelines, 2012. [7]

## 2 Why are blood cultures important?

Blood culture is the most widely used diagnostic tool for the detection of bacteremia and fungemia. It is the most important way to diagnose the etiology of bloodstream infections and sepsis and has major implications for the treatment of those patients.

A positive blood culture either establishes or confirms that there is an infectious etiology for the patient's illness. [3] A positive blood culture also provides the etiologic agent for antimicrobial susceptibility testing, enabling optimization of antibiotic therapy. [3] Sepsis is one of the most significant challenges in critical care, and early diagnosis is one of the most decisive factors in determining patient outcome. Early identification of pathogens in the blood can be a crucial step in assuring appropriate therapy, and beginning





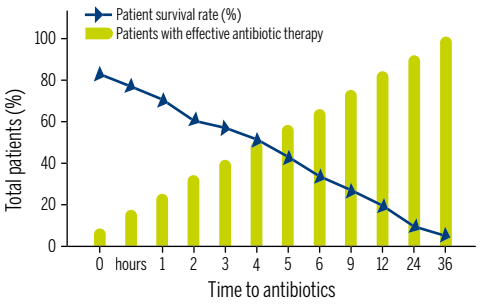
effective antibiotic therapy as early as possible can have a significant impact on the outcome of the disease. [8,9]

### → Providing adequate antibiotic therapy within the first 24-48 hours leads to: [10-14]

- Decreased infection-related mortality (20-30%)
- Earlier recovery and shorter length of hospital stay
- Less risk of adverse effects
- Reduced risk of antimicrobial resistance
- Cost reduction (length of stay, therapy, diagnostic testing)

**Figure 1: Fast effective antimicrobial therapy increases survival chances**

Adapted from Kumar A, *et al.* Crit Care Med. 2006;34(6):1589-96. [15]




## 3 When should a blood culture be performed?

Blood cultures should always be requested when a bloodstream infection or sepsis is suspected.

### → Clinical symptoms in a patient which may lead to a suspicion of a bloodstream infection are:

- undetermined fever (≥38°C) or hypothermia (≤36°C)
- shock, chills, rigors
- severe local infections (meningitis, endocarditis, pneumonia, pyelonephritis, intra-abdominal suppuration...).
- abnormally raised heart rate
- low or raised blood pressure
- raised respiratory rate

### → Blood cultures should be collected:

- as soon as possible after the onset of clinical symptoms;
- ideally, prior to the administration of antimicrobial therapy [16].

If the patient is already on antimicrobial therapy, recovery of microorganisms may be increased by collecting the blood sample immediately before administering the next dose and by inoculating the blood into bottles containing specialized antimicrobial neutralization media.

## 4 What volume of blood should be collected?

The optimal recovery of bacteria and fungi from blood depends on culturing an adequate volume of blood. The collection of a sufficient quantity of blood improves the detection of pathogenic bacteria or fungi present in low quantities. This is essential when an endovascular infection (such as endocarditis) is suspected.

> **The volume of blood that is obtained for each blood culture set is the most significant variable in recovering microorganisms from patients with bloodstream infections.** [17, 18]

Blood culture bottles are designed to accommodate the recommended blood-to-broth ratio (1:5 to 1:10) with optimal blood volume. Commercial continuously monitoring blood culture systems may use a smaller blood-to-broth ratio (< 1:5) due to the addition of sodium polyanetholesulfonate (SPS) which inactivates inhibitory substances which are present in blood. [3]

### → Adults

**For an adult, the recommended volume of blood to be obtained per culture is 20 to 30 ml.** [3, 16]

**Since each set includes an aerobic and an anaerobic bottle**, each bottle should be inoculated with approximately 10 ml of blood. This volume is recommended to optimize pathogen recovery when the bacterial/fungal burden is less than 1 Colony Forming Unit (CFU) per ml of blood, which is a common finding.





It is also generally recommended that **two or three bottle sets** (two bottles per set) are used per septic episode, meaning, for adults, 40 to 60 ml of blood collected from the patient for the 4 to 6 bottles, with 10 ml per bottle.

For each additional milliliter of blood cultured, the yield of microorganisms recovered from adult blood increases in direct proportion up to 30 ml. [19] This correlation is related to the relatively low number of CFU in a milliliter of adult blood. [3]

### → Pediatric

The optimal volume of blood to be obtained from infants and children is less well prescribed, however, available data indicate that the yield of pathogens also increases in direct proportion to the volume of blood cultured. [16, 20] The recommended volume of blood to collect should be **based on the weight of the patient** (see Table 1), and an aerobic bottle should be used, unless an anaerobic infection is suspected. [21]

Specially formulated blood culture bottles are commercially available for use in children <2 years of age. They are specifically designed to maintain the usual blood-to-broth ratio (1:5 to 1:10) with smaller blood volumes, and have been shown to improve microbial recovery. [3]

**Table 1: Blood volumes suggested for cultures from infants and children** [20]

Adapted from Kellogg *et al*. Frequency of low-level bacteremia in children from birth to fifteen years of age. J Clin Microbiol. 2000; 38:2181-2185.

| Weight of patient | | Patient's total blood volume (ml) | Recommended volume of blood for culture (ml) | | Total volume for culture (ml) | % of patient's total blood volume |
|---|---|---|---|---|---|---|
| kg | lb | | Culture no.1 | Culture no.2 | | |
| ≤1 | ≤2.2 | 50-99 | 2 | | 2 | 4 |
| 1.1-2 | 2.2-4.4 | 100-200 | 2 | 2 | 4 | 4 |
| 2.1-12.7 | 4.5-27 | >200 | 4 | 2 | 6 | 3 |
| 12.8-36.3 | 28-80 | >800 | 10 | 10 | 20 | 2.5 |
| >36.3 | >80 | >2,200 | 20-30 | 20-30 | 40-60 | 1.8-2.7 |

BLOOD CULTURE ESSENTIALS

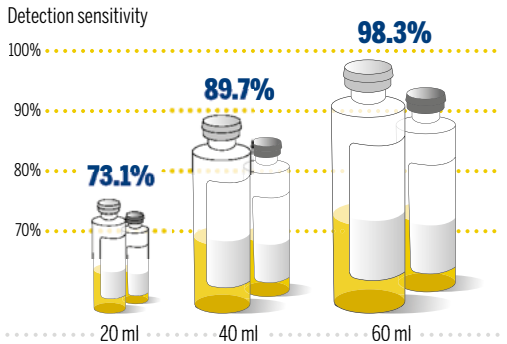
# 5 How many blood culture sets should be collected?

Since bacteria and fungi may not be constantly present in the bloodstream, **the sensitivity of a single blood culture set is limited**.

Using continuous-monitoring blood culture systems, a study investigated the cumulative sensitivity of blood cultures obtained sequentially over a 24-hour time period. It was observed that the cumulative yield of pathogens from three blood culture sets (2 bottles per set), with a blood volume of 20 ml in each set (10 ml per bottle), was 73.1% with the first set, 89.7% with the first two sets and 98.3% with the first three sets. However, to achieve a detection rate of >99% of bloodstream infections, as many as four blood culture sets may be needed.[22]

**Figure 2: Cumulative sensitivity of blood culture sets**[22]

Adapted from Lee *et al*. Detection of Bloodstream Infections in Adults: How Many Blood Cultures Are Needed? J Clin Microbiol. 2007; 45:3546-3548




**A single blood culture bottle or set should never be drawn from adult patients, since this practice will result in an inadequate volume of blood cultured and a substantial number of bacteremias may be missed.**[3, 22]

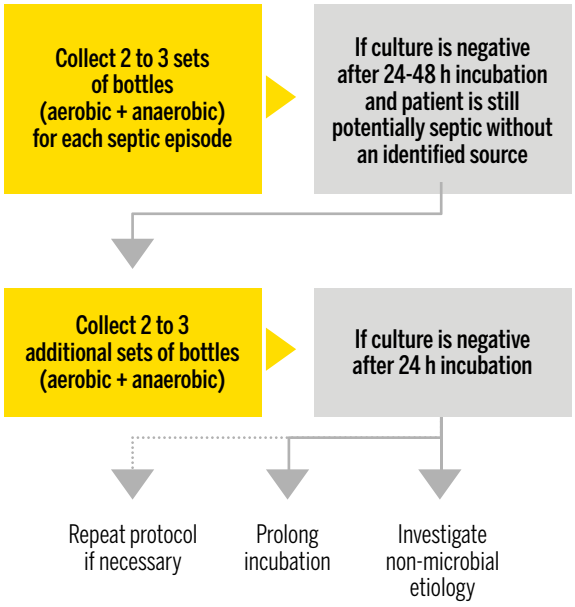
A contaminant will usually be present in only one bottle of a set of blood culture bottles, in contrast to a true bloodstream infection, in which multiple blood culture bottles/sets will be positive.

**Therefore, guidelines recommend to collect 2, or preferably 3, blood culture sets for each septic episode.**[3, 7, 16]

If 2 to 3 sets are taken and cultures are still negative after 24-48 hours incubation, and the patient is still potentially septic, 2 to 3 additional cultures may be collected, as indicated in the following diagram.[16]

**Figure 3: Recommended number of blood culture sets**

Adapted from Baron, E.J., *et al*. Cumitech 1C, Blood Cultures IV. Coordinating ed., E.J. Baron. ASM Press, Washington, D.C. 2005



# 6 Which media to use?

Microorganisms causing bloodstream infections are highly varied (aerobes, anaerobes, fungi, fastidious microorganisms...) and, in addition to nutrient elements, may require specific growth factors and/or a special atmosphere.

In cases where the patient is receiving antimicrobial therapy, specialized media with antibiotic neutralization capabilities should be used. **Antibiotic neutralization media** have been shown to increase recovery and provide faster time to detection *versus* standard media.[23-26]

**It is recommended that each adult routine blood culture set include paired aerobic and anaerobic blood culture bottles.**

**The blood drawn should be divided equally between the aerobic and anaerobic bottles.**

**If an anaerobic bottle is not used, it should always be replaced by an additional aerobic bottle to ensure that a sufficient volume of blood is cultured.**[27]

## → A blood culture medium must be:

- **sensitive** enough to recover:
  - a broad range of clinically relevant microorganisms, even the most fastidious (*Neisseria, Haemophilus*...)
  - microorganisms releasing small amounts of $CO_2$ (*Brucella, Acinetobacter*...)
- **versatile:** able to provide a result for all types of sample collection (adults, infants, patients receiving antibiotic therapy, sterile body fluids...)

## → Which bottle should be inoculated first?

If using a **winged blood collection set**, then the **aerobic bottle should be filled first** to prevent transfer of air in the device into the anaerobic bottle.

If using a **needle and syringe**, inoculate the **anaerobic bottle first** to avoid entry of air.

If the amount of blood drawn is less than the recommended volume*, then approximately 10 ml of blood should be inoculated into **the aerobic bottle first**, since most cases of bacteremia are caused by aerobic and facultative bacteria. In addition, pathogenic yeasts and strict aerobes (e.g. *Pseudomonas*) are recovered almost exclusively from aerobic bottles. Any remaining blood should then be inoculated into the anaerobic bottle.[8]

* For recommended volumes, see page 6 "What volume of blood should be collected?





# 7 Timing of blood cultures

Studies have shown that the time interval between collecting two blood culture samples is not considered to be a critical factor as the diagnostic yield remains the same.[7]

Guidelines recommend that the **first two/three sets (2 bottles/set) of blood culture** be obtained either **over a brief time period (e.g. within 1 hour)** or as **a single sample taken at one time.**[3, 7, 16] The possible impact that the blood culture collection method used (e.g. single or multiple venipunctures, winged collection set or needle and syringe) may have on contamination rates should be considered.[7]

Drawing blood at spaced intervals, such as 1 to 2 hours apart, is only recommended to monitor continuous bacteremia/fungemia in patients with suspected infective endocarditis or other endovascular (i.e. catheter- related) infections.[16]

Two to three additional blood culture sets can be performed if the first 2-3 blood cultures are negative after 24-48 hours incubation in cases of severe infection or in order to increase detection sensitivity (in cases of pyelonephritis for example). This also depends on the microorganisms involved: while sensitivity is relatively good for organisms like *Escherichia coli* or *Staphylococcus aureus*, it is lower for *Pseudomonas aeruginosa*, streptococci or fungi.[28]

# 8 How to collect blood cultures

Sample collection is a crucial step in the blood culture process. Standard precautions must be taken, and strict aseptic conditions observed throughout the procedure. Compliance with blood culture collection recommendations can significantly improve the quality and clinical value of blood culture investigations and reduce the incidence of sample contamination and "false-positive" readings.

**A properly collected sample, that is free of contaminants, is key to providing accurate and reliable blood culture results.**

It is recommended that blood cultures should be collected only by members of staff (medical, nursing, phlebotomist or technician) who have been fully trained and whose competence in blood culture collection has been assessed.[29]

## 10 Key Steps to Good Sample Collection:

For an illustrated step-by-step, see page 30.

1. Prior to use, **examine the bottles** for evidence of damage, deterioration or contamination. Do not use a bottle containing media which exhibits turbidity or excess gas pressure, as these are signs of possible contamination.
2. **Check the expiry date** printed on each bottle. Discard bottles that have expired.
3. **Strictly follow the collection protocol** in use in the healthcare setting, including standard precautions for handling blood at the bedside.
4. Blood culture bottles should be **clearly and correctly labelled**, including patient identification, date and collection time, puncture site (venipuncture or intravascular device).
5. Each blood culture set should include **an aerobic and an anaerobic bottle**.
6. Blood for culture should be **drawn from veins, not arteries**.[30]
7. It is recommended to **avoid drawing blood from a venous or arterial catheter**, since these devices are often associated with higher contamination rates.[31]
8. **Carefully disinfect the skin** prior to collection of the sample using an appropriate disinfectant, such as chlorhexidine in 70% isopropyl alcohol or tincture of iodine in swab or applicator form.[3]
9. **Transport the inoculated bottles** and the completed blood culture request to the clinical microbiology laboratory as quickly as possible, preferably within 2 hours per CLSI.[3]

   Any delay in testing the inoculated bottles may potentially lead to an increased risk of false negative results. If delays are expected, it is important to refer to the manufacturer's Instructions for Use (IFU) for guidance.

   As an example for guidance regarding delays, the ESCMID guidelines recommend that blood culture bottles for testing in continuous monitoring systems should be stored temporarily at room temperature, whereas bottles for manual testing should be incubated as soon as possible.[32] Again, refer to the manufacturer's IFU for guidance.

   The use of vacuum tube transport systems can facilitate the rapid transmission of bottles to the microbiology laboratory. However these systems should be used with caution if using glass bottles.[33]
10. **All blood cultures should be documented** in the patient's notes, including date, time, collection site and indications.



BLOOD CULTURE ESSENTIALS

# 9 How many days of incubation are recommended?

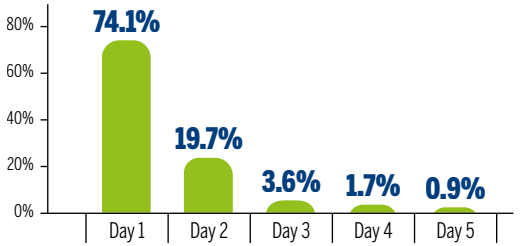
**The current recommendation, and standard incubation period, for routine blood cultures performed by continuous-monitoring blood systems is five days.** [34]

However, published data suggest that **three days may be adequate** to recover over 97% of clinically significant microorganisms.

A study by Bourbeau, *et al*. (JCM, 2005) showed the number of significant microorganisms isolated per day for 35,500 consecutive blood cultures collected over 30 months, of which 2,609 were clinically significant isolates and 1,097 were contaminants. [35]

**Figure 4: Clinically significant isolates per day** [35]

Adapted from Bourbeau PP *et al*. Routine incubation of BacT/ALERT® FA and FN blood culture bottles for more than 3 days may not be necessary. J Clin Microbiol. 2005;43:2506-2509




These results demonstrate that 97.4% of clinically significant isolates were recovered within the first 3 days of incubation and 93.8% within 2 days of incubation.

## → Incubation of Fastidious Microorganisms

Another study by Cockerill, *et al*. (CID, 2004) demonstrated that, when using a continuous-monitoring blood culture system, 99.5% of non-endocarditis bloodstream infections and 100% of endocarditis episodes were detected within 5 days of incubation. [19] This data suggests that extended incubation periods previously recommended for detection of the fastidious micro-organisms* that sometimes cause endocarditis, are no longer necessary when using continuous-monitoring blood culture systems. [16]

* including *Brucella*, *Capnocytophaga* and *Campylobacter* spp., and the HACEK group (*Haemophilus* (except *H. influenzae*) species, *Aggregatibacter* (previously *Actinobacillus*) species, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* species) [36]





# 10 Is it a contaminant or a true pathogen?

Contamination of blood cultures during the collection process can produce a significant level of false-positive results, which can have a negative impact on patient outcome.

A **false positive** is defined as growth of bacteria in the blood culture bottle that were not present in the patient's bloodstream, and were most likely introduced during sample collection.

Contamination can come from a number of sources: the patient's skin, the equipment used to take the sample, the hands of the person taking the blood sample, or the environment.

**Collecting a contaminant-free blood sample is critical to providing a blood culture result that has clinical value.**

Certain microorganisms such as coagulase-negative staphylococci, viridans-group streptococci, *Bacillus* spp, *Propionibacterium* spp., diphtheroids, *Micrococcus* spp. rarely cause severe bacterial infections or bloodstream infections. These are **common skin contaminants**, and a though they are capable of causing serious infection in the appropriate setting, their detection in a single blood culture set can reasonably be identified as a possible contaminant without clinical significance. However, it is important to consider that coagulase-negative staphylococci are the primary cause of both catheter- and prosthetic device-associated infections and may be clinically significant in up to 20% of cases. [37]

The most difficult interpretation problem for the physician is whether the organism recovered from a blood culture is a **true pathogen causing bloodstream infection**, or a **contaminant**. If it is a contaminant, the patient may be treated unnecessarily with antibiotics, leading to additional patient risks. Interpretation of true pathogen *versus* contaminant should be based on whether the blood has been collected with a venipuncture or an intra-vascular device, and multiplicity of isolation of the same species. This illustrates the crucial nature of having **collection site information included with the blood culture request sent to the laboratory**.

In contrast to patients with infective endocarditis or other true positive bloodstream infections, patients whose blood cultures grow contaminants usually have only a single blood culture that is positive. This information is of great practical value for physicians, and underlines the importance of taking two to three blood culture sets from different anatomical sites.[16]

**Contamination rates can be most effectively reduced by strict compliance with hand hygiene rules and best practices for blood collection, particularly during the stages of skin antisepsis, venipuncture and sample transfer to blood culture bottles.**

However, even when the best blood collection protocols are used, it may not be possible to reduce the contamination rate below 2%.[38] The American Society for Microbiology and CLSI recommend targeting contamination rates not exceeding 3% of the total of collected sets.[3,16]

## Impact of contamination rates

A contaminated blood culture can result in unnecessary antibiotic therapy, increased length of hospitalization and higher costs.

It has been found that each false positive result can lead to:

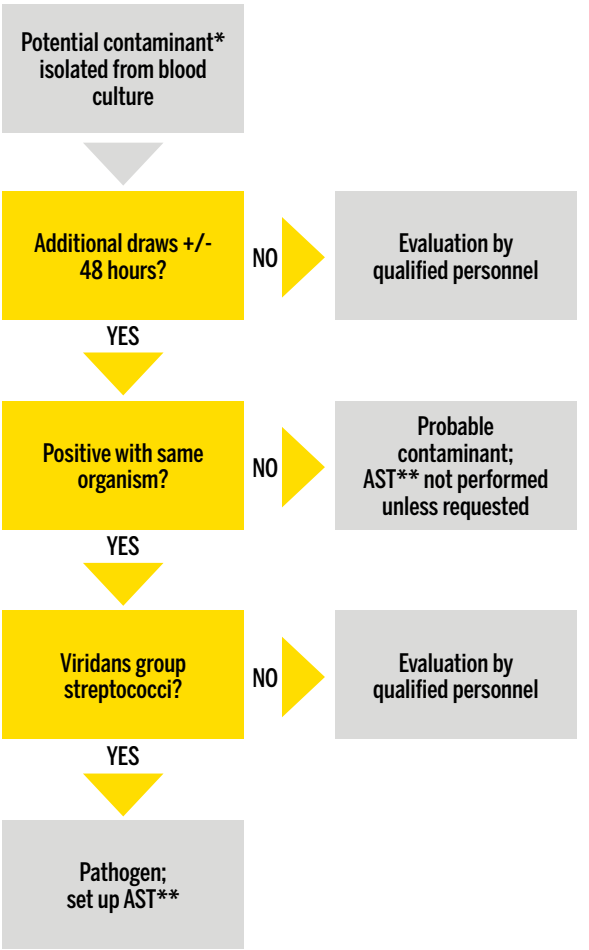
- Increased length of stay - on average 1 day.[39]
- 39% increase in intravenous antibiotic charges.[39]
- $5,000 to $8,720 additional charges.[40,41]
- 20% increase in laboratory charges.[39]
- 3 days longer on antibiotics.[39]



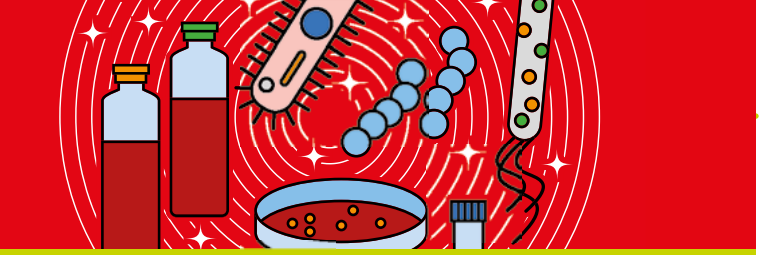


**Figure 5: Example of a laboratory-based algorithm to determine blood culture contamination[42]**

Adapted from Richter *et al.* Minimizing the workup of blood culture contaminants: implementation and evaluation of a laboratory-based algorithm. J Clin Microbiol. 2002;40:2437-2444.




* Microorganisms such as coagulase-negative staphylococci, *Streptococcus viridans*, *Bacillus* spp, *Propionibacterium* spp., diphtheroids, *Micrococcus* spp.
** AST: Antimicrobial Susceptibility Testing





# 2 SPECIAL TOPIC: INFECTIVE ENDOCARDITIS

**Blood culture is essential in the diagnosis of infective endocarditis (infection of the heart valves). In this elusive disease, blood cultures may need to be taken repeatedly during febrile episodes, when bacteria are shed from the heart valves into the bloodstream. For patients with infective endocarditis, positive blood cultures will be obtained in over 90% of cases, if optimal culture conditions are respected.** [43]

## → Acute Infective Endocarditis

This is a fulminant illness progressing rapidly over days to weeks, which may be caused by highly virulent pathogens, such as *Staphylococcus aureus*. When suspected, the severity of this disease requires blood cultures to be drawn immediately to avoid unnecessary delays in treatment.

- Multiple blood culture sets should be drawn during a 30-minute period prior to administration of empiric antimicrobial therapy. [44]

## → Subacute Infective Endocarditis

If sub-acute infection is suspected, there is usually not an urgent need to initiate empiric therapy. It is more important to attempt to establish the microbiological diagnosis.

- Multiple blood culture sets should be obtained prior to initiation of antimicrobial therapy, with sets spaced 30 minutes to one hour apart. This may help document a continuous bacteremia, and could be of additional clinical value. [3]

## → Fungal Infective Endocarditis

Once a rare occurrence, the incidence of fungal endocarditis is increasing considerably. [45] *Candida* species are the most common fungal pathogens involved in infective endocarditis. [46] If optimum collection conditions are observed, the yield for positive blood cultures in fungal endocarditis for *Candida* spp. is 83 to 95 %. [47]

## → How many cultures?

In order to distinguish between contamination and true bacteremia, a total of three to five blood culture sets should be sufficient.

- Initially, two to three blood culture sets should be obtained from patients with suspected infective endocarditis. If the first 2-3 sets are negative after 24-48 hours, collect two to three more sets of cultures. [3]

Often patients with suspected infective endocarditis have been put on antibiotics prior to blood collection. This is the most common reason for **"culture-negative" infective endocarditis**. It is therefore important to use a blood culture medium that has antimicrobial neutralization capacity in order to sustain microbial growth in the presence of antibiotics (see page 10 "Which media to use?"). [48, 49]

However, "culture-negative" endocarditis may also be due to fastidious microorganisms, such as *Aspergillus* spp., *Brucella* spp., *Coxiella burnetii*, *Chlamydia* spp. and HACEK* microorganisms.

- Since current continuous-monitoring blood culture systems can recover all HACEK and other fastidious organisms within a 5-day period, extending incubation beyond this period is no longer considered to be necessary. However, if all blood culture bottles are negative after 5 days, and infectious endocarditis is still suspected, all bottles should be subcultured to chocolate agar. [50]



# 3 PROCESSING POSITIVE BLOOD CULTURES

Today, continuously-monitored blood culture systems provide the optimum solution for blood sample processing. Generally accepted incubation periods can vary from 5-7 days, with 5 days being most popular.[27] The study discussed in Figure 4 shows that 98% of all positive specimens were detected within the first 3 days (see page 14).[35]

**Patients who progress to septic shock have a 7.6% increase in mortality every hour while not on appropriate therapy.**[15]

Following an instrument-flagged positive event, the bottle is removed from the system and a Gram stain and subculture is performed.

- **If the sample is Gram stain positive**, the morphology of the organism should be reported immediately to the physician. Subcultures or rapid techniques (e.g. molecular diagnostics) should be initiated immediately in order to provide further organism identification and antibiotic susceptibility testing should be performed as soon as possible.
- **If a sample is Gram stain negative**, no report is made to the clinician unless there is growth on subculture.

A positive blood culture is a critical result and must be reported as soon as available, due to the immediate impact on patient care decisions. When **reports are delivered rapidly**, studies have shown broadly improved outcomes and efficiencies in patient management.[51, 52]

A study by Barenfanger, *et al*. (Am J Clin Pathol, 2008) validated that Gram stains of positive blood cultures are a very important factor influencing **appropriate therapy and patient outcomes**. The study documented a statistically significant increase in the mortality rate for patients who had blood cultures processed after a delay (i.e. Gram stain performed ≥1 hour after being detected as positive; P = 0.0389). The timely removal and reporting of Gram stain results have a positive impact on patient care and this study supports the need for 24/7 coverage of blood culture instruments.[53]

Recent technological advances such as **MALDI-TOF** (Matrix-Assisted Laser Desorption Ionization Time of Flight) provide the ability to rapidly deliver definitive organism identification. **Molecular diagnostics** can identify the most common pathogens in positive blood cultures as well as specific antibiotic resistance genes associated with bloodstream infections. Rapid identification allows physicians to prescribe more targeted and effective antimicrobial therapy earlier to positively influence outcomes.[54-56]

Additionally, **antibiotic susceptibility testing** techniques should be performed on positive blood cultures to provide the clinician with a complete result. Appropriate use of antibiotics is crucial in cases of bloodstream infections and sepsis. Accurately determining the antimicrobial resistance profile of the causative pathogen in order to select the most effective antibiotic therapy can have a significant impact on patient outcomes.

**When processed correctly, blood cultures provide clinically relevant information that can help improve patient outcomes, decrease length of hospital stay and reduce use of antibiotics.**

# 4 INTERPRETATION OF RESULTS

The microbiology laboratory can provide useful information to clinicians to help them determine whether a blood culture sample is a true positive or a false positive (contaminant). For example, the identity of the micro- organism isolated can help determine if the culture is contaminated, and the number of

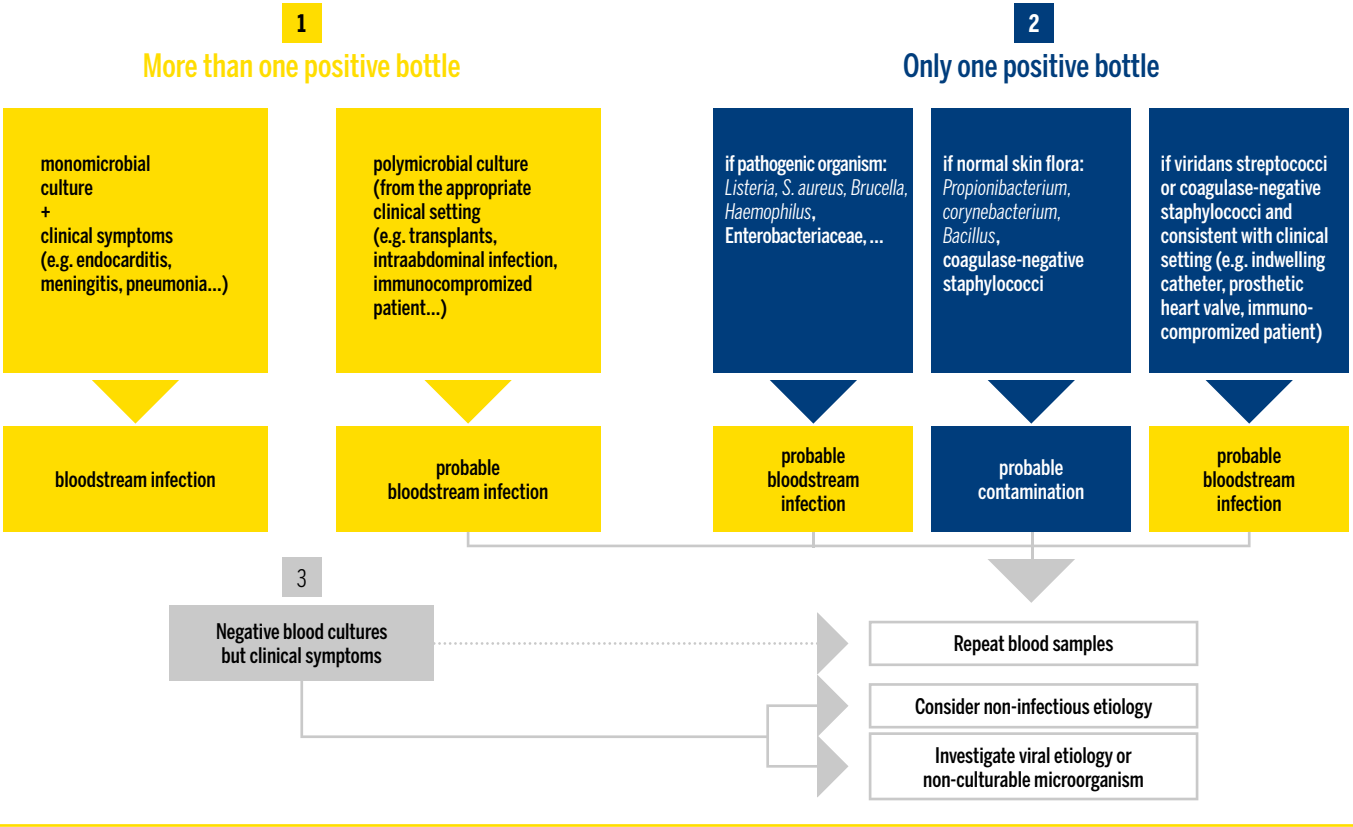


cultures positive with the same organism can help predict true infections.[57] Time to positivity is also a factor used to determine potential contamination as contaminants usually have a delayed (longer) time-to-detection due to a lower overall bio-load.

Laboratories should consult with their medical director to create an algorithm which helps determine whether or not an isolated organism is a contaminant vs. an infective agent.

Models, such as the algorithm below, can give **guidance only on the interpretation of blood culture results**.[42, 57, 58] These guidelines should be used in conjunction with clinical guidelines, e.g. patient's full blood count, presence of catheters, radiological findings, etc.

**Figure 6: Example of interpretation algorithm for blood culture results**



# 5 BLOOD CULTURE/SEPSIS GUIDELINES

## ➔ International Guidelines

**WHO guidelines on drawing blood: best practices in Phlebotomy.**
World Health Organization 2010.
*http://whqlibdoc.who.int/publications/ 2010/9789241599221_eng.pdf*

**Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012.**
Dellinger RP., *et al*. Crit Care Med. 2013;41:580-637.
*http://www.survivingsepsis.org/guidelines/Pages/default.aspx*

**The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).**
Singer M., *et al.* JAMA. 2016;315(8):801-810.
*http://jama.jamanetwork.com/article.aspx?articleid=2492881*

## ➔ National Guidelines

| COUNTRY/ REGION | GUIDELINES |
|---|---|
| Australia | Australia Clinical Excellence Commission Sepsis Kills Program: Adult Blood Culture Sampling Guide v2 2012 SHPN (CEC) 120077<br>*http://www.cec.health.nsw.gov.au/__data/assets/pdf_file/0005/259412/adult-blood-culture-sampling-guideline.pdf* |
| Brazil | Elmor de Araujo MR, Hemocultura: recomendações de coleta, processamento e interpretação dos resultados, J Infect Control 2012; 1: 08-19<br>*http://www.iqg.com.br/pbsp/img_up/01355393320.pdf* |
| Europe | European Society for Clinical Microbiology and Infectious Diseases, European Manual for Clinical Microbiology, 1st Edition, 2012.<br>*https://www.escmid.org/escmid_library/manual_of_microbiology/* |





| COUNTRY/ REGION | GUIDELINES |
|---|---|
| France | REMIC 2015. Automatisation des cultures microbiennes : quel cahier des charges ? Chapitre 11<br>*http://www.sfm-microbiologie.org/* |
| Germany | Reinhart K et *al.*, Prevention, diagnosis, therapy and follow-up care of sepsis: 1st revision of S-2k guidelines of the German Sepsis Society (Deutsche Sepsis-Gesellschaft e.V. (DSG)) and the German Interdisciplinary Association of Intensive Care and Emergency Medicine (DIVI). German Medical Science, 2010, Vol. 8: 1-86<br>*http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2899863/pdf/GMS-08-14.pdf* |
| South Africa | Guideline for the optimal use of blood cultures. SAMJ 2010; Vol. 100, No. 12: 839-843 SAMJ<br>*http://www.fidssa.co.za/Guidelines/Guideline_for_the_optimal_ use_of_blood_cultures.pdf* |
| UK | ■ UK Standards for Microbiology Investigations. Investigation of Blood Cultures (for Organisms other than Mycobacterium species). Bacteriology \| B 37 \| Issue no: 8 \| Issue date: 04.11.14 \| Page: 1 of 51. Issued by the Standards Unit, Health Protection Agency, PHE.<br>*https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/372070/B_37i8.pdf*<br>■ Taking blood cultures - a summary of best practice: Saving lives reducing infection, delivering clean and safe care. London: Department of Health; 2007.<br>*http://webarchive.nationalarchives.gov.uk/20120118164404/hcai.dh.gov.uk/files/2011/03/Document_Blood_culture_FINAL_100826.pdf* |
| USA | ■ American Society for Microbiology: Cumitech 1C, 2005 (EJ Baron *et al*.) ASM Press<br>■ Clinical and Laboratory Standards Institute (CLSI®), document M47-A, Vol 27, 2007 (ML Wilson *et al*.)<br>■ Emergency Nurses Association (ENA). Clinical Practice Guideline: Prevention of Blood Culture Contamination<br>*https://www.ena.org/practice-research/research/CPG/Documents/BCCCPG.pdf*<br>■ E. Septimus. CDC Clinician Guide for Collecting Cultures. 2015<br>*http://www.cdc.gov/getsmart/healthcare/implementation/clinicianguide.html* |

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3. WSD fact sheet 2013/www.world-sepsis-day.org
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5. Singer M., *et al.* The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-810
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# RECOMMENDATIONS FOR BLOOD CULTURE COLLECTION

## A SUMMARY OF GOOD PRACTICE

### A) USING WINGED BLOOD COLLECTION SET

(preferred method of collection)[59, 60, 61]

#### 1 PREPARE BLOOD COLLECTION KIT

**Confirm the patient's identity** and gather all required materials before beginning the collection process.

**Do not use blood culture bottles beyond their expiration date**, or bottles which show signs of damage, deterioration or contamination.

It is recommended to identify the Fill-to Mark or mark the target fill level on the blood culture bottle label about 10 ml above the media level.

#### 2 PREPARE BOTTLES FOR INOCULATION

Wash hands with soap and water then dry, or apply an alcohol hand rub or another recognized effective hand rub solution.

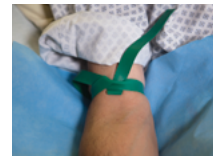
Remove the plastic "flip-cap" from the blood culture bottles and disinfect the septum using an appropriate and recognized effective disinfectant, such as chlorhexidine in 70% isopropyl alcohol, 70% isopropyl alcohol, or tincture of iodine in swab or applicator form. Use a fresh swab/applicator for each bottle.

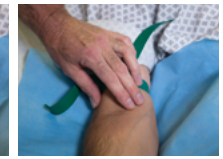
**Allow bottle tops to dry in order to fully disinfect.**

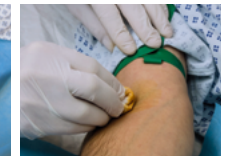
#### 3 PREPARE VENIPUNCTURE SITE

If skin is visibly soiled, clean with soap and water. Apply a disposable tourniquet and palpate for a vein. **Apply clean examination gloves** (sterile gloves are not necessary).

Cleanse the skin using an appropriate disinfectant, such as chlorhexidine in 70% isopropyl alcohol or tincture of iodine in swab or applicator form. **The venipuncture site is not fully clean until the disinfectant has fully evaporated.**



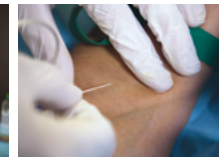


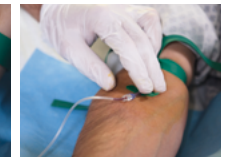


#### 4 VENIPUNCTURE

Attach a winged blood collection set to a collection adapter cap*.

**To prevent contaminating the puncture site, do not re-palpate the prepared vein before inserting the needle.** Insert the needle into the prepared vein.





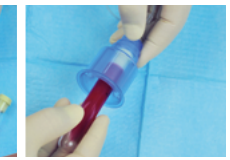
#### 5 CULTURE BOTTLE INOCULATION

Place the adapter cap over the **aerobic bottle** and **press straight down** to pierce the septum. Hold the bottle upright, below the level of the draw site, and add up to 10 ml of blood per adult bottle and up to 4 ml per pediatric bottle.** Ensure the bottle is correctly filled to the Fill-to Mark or target fill level. Once the aerobic bottle has been inoculated, repeat the procedure for the **anaerobic bottle**.

#### 6 OTHER BLOOD TESTS

If blood is being collected for other tests, an insert placed into the adapter cap may be required. The insert is used to guide blood collection tubes onto the needle.

**If other blood tests are requested, always collect the blood culture first.**

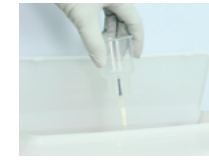


#### 7 FINISH THE PROCEDURE

Discard the winged collection set into a sharps container and cover the puncture site with an appropriate dressing. Remove gloves and wash hands before recording the procedure, including indication for culture, date, time, site of venipuncture, and any complications.

**Ensure additional labels are placed in the space provided on the bottle label and do not cover the bottle barcodes, and that the tear-off barcode labels are not removed.** If additional labels contain a barcode, they should be positioned in the same manner as the bottle barcode.

Inoculated bottles should be transported to the laboratory for testing as quickly as possible, preferably within 2 hours per CLSI.[3] If delays are expected, it is important to refer to the manufacturer's Instructions for Use for guidance.



** The use of blood collection sets without blood collection adapters is not recommended.*

***Avoid holding the blood culture bottle in a horizontal or upside down position or drawing blood with a needle connected directly to the adaptor cap, as fill level cannot be monitored during collection and there is a possible risk of media reflux into the bloodstream.*

**These recommendations illustrate the best practices for blood culture collection based on the World Health Organization recommendations (WHO guidelines on drawing blood: best practices in phlebotomy. 2010. ISBN 978 92 4 159922 1). Best practices may vary between healthcare facilities; refer to guidelines applicable in your facility.**

# RECOMMENDATIONS FOR BLOOD CULTURE COLLECTION

## A SUMMARY OF GOOD PRACTICE

## B) USING NEEDLE AND SYRINGE

Conventional needles and syringes should be replaced wherever possible with winged blood collection sets, which are safer.[59, 60, 61]

They should only be used if prevention measures to Accidental Blood Exposure are strictly applied*. Needles must not be recapped, purposely bent or broken by hand, removed from disposable syringes or otherwise manipulated by hand.

### 1 PREPARE BLOOD COLLECTION KIT

**Confirm the patient's identity** and gather all required materials before beginning the collection process.
**Do not use blood culture bottles beyond their expiration date**, or bottles which show signs of damage, deterioration or contamination.
It is recommended to identify the Fill-to Mark or mark the target fill level on the blood culture bottle label about 10 ml above the media level.

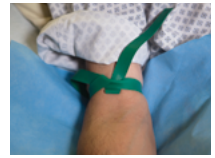
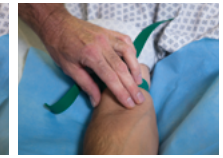
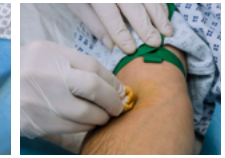



### 2 PREPARE BOTTLES FOR INOCULATION

Wash hands with soap and water then dry, or apply an alcohol hand rub or another recognized effective hand rub solution.
Remove the plastic "flip-cap" from the blood culture bottles and disinfect the septum using an appropriate and recognized effective disinfectant, such as chlorhexidine in 70% isopropyl alcohol, 70% isopropyl alcohol, or tincture of iodine in swab or applicator form. Use a fresh swab/applicator for each bottle.
**Allow bottle tops to dry in order to fully disinfect.**

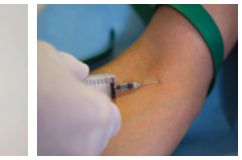

### 3 PREPARE VENIPUNCTURE SITE

If skin is visibly soiled, clean with soap and water. Apply a disposable tourniquet and palpate for a vein. **Apply clean examination gloves** (sterile gloves are not necessary).
Cleanse the skin using an appropriate disinfectant, such as chlorhexidine in 70% isopropyl alcohol or tincture of iodine in swab or applicator form. **The venipuncture site is not fully clean until the disinfectant has fully evaporated.**

### 4 VENIPUNCTURE

Attach the needle to a syringe. **To prevent contaminating the puncture site, do not re-palpate the prepared vein before inserting the needle.**

Insert the needle into the prepared vein.

### 5 CULTURE BOTTLE INOCULATION

Collect the sample. Transfer the blood into the culture bottles, starting with the **anaerobic bottle**. Hold the bottle upright, and add up to 10 ml of blood per adult bottle and up to 4 ml per pediatric bottle. Ensure the bottle is correctly filled to the Fill-to Mark or target fill level. Once the anaerobic bottle has been inoculated, repeat the procedure for the **aerobic bottle**.



### 6 FINISH THE PROCEDURE

Discard the needle and syringe into a sharps container and cover the puncture site with an appropriate dressing. Remove gloves and wash hands before recording the procedure, including indication for culture, date, time, site of venipuncture, and any complications.
**Ensure additional labels are placed in the space provided on the bottle label and do not cover the bottle barcodes, and that the tear-off barcode labels are not removed.** If additional labels contain a barcode, they should be positioned in the same manner as the bottle barcode. Inoculated bottles should be transported to the laboratory for testing as quickly as possible, preferably within 2 hours per CLSI.[3] If delays are expected, it is important to refer to the manufacturer's Instructions for Use for guidance.


** Refer to recognized guidelines such as those issued by the WHO or CDC:*
*http://www.who.int/injection_safety/phleb_final_screen_ready.pdf*
*http://www.cdc.gov/niosh/docs/2000-108/pdfs/2000-108.pdf*

**These recommendations illustrate the best practices for blood culture collection based on the World Health Organization recommendations (WHO guidelines on drawing blood: best practices in phlebotomy. 2010. ISBN 978 92 4 159922 1). Best practices may vary between healthcare facilities; refer to guidelines applicable in your facility.**

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# Monoclonal Mouse Anti-Human Cytokeratin 7
Clone OV-TL 12/30
**Code M7018**

## ENGLISH

Techninės specifikacijos 31 reikalavimas

**Intended use**
For in vitro diagnostic use.

Monoclonal Mouse Anti-Human Cytokeratin 7, Clone OV-TL 12/30, is intended for use in immunohistochemistry (IHC). The antibody labels glandular and transitional epithelial cells and is a useful aid for the classification of adenocarcinoma of the lung (1, 2), breast and endometrium, thyroid gland (1, 3) and ovary (1, 4), as well as transitional cell (urothelial) carcinoma (1), and chromophobe renal cell carcinoma (5). Differential classification is aided by the results from a panel of antibodies. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

**Summary and explanation**
Cytokeratin 7 belongs to the intermediate filaments, which create a cytoskeleton in almost all eukaryotic cells. In contrast to other intermediate filaments, cytokeratins (CKs) are made up of a highly complex multigene family of polypeptides with molecular masses ranging from 40 to 68 kDa. CKs are generally held to belong to the most fundamental markers of epithelial differentiation, and until now, 20 distinct CK polypeptides have been revealed in various human epithelia (6, 7). The CKs can be divided into an acidic type A (class I) and a neutral-basic type B (class II) subfamily. CK7, a 54 kDa protein, belongs to the neutral-basic type B subfamily, and its distribution is confined to glandular and transitional epithelia (6).

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure; Materials Required, Not Supplied; Storage; Specimen Preparation; Staining Procedure; Quality Control; Troubleshooting; Interpretation of Staining; General Limitations.

**Reagent provided**
Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 0.05 mol/L Tris-HCL, pH 7.2, and containing 15 mmol/L $NaN_3$.

Clone: OV-TL 12/30 (1). Isotype: IgG1, kappa.

Mouse IgG concentration: see label on vial.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

**Immunogen**
OTN 11, ovarian carcinoma cell line (1, 8).

**Specificity**
In one- and two-dimensional SDS-PAGE and immunoblotting of Triton X-100-extracted cytoskeleton preparations of several cell cultures and tissues, the antibody labeled a 54 kDa band corresponding to cytokeratin 7 (1).

**Precautions**
1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution should be disposed of according to local, State and Federal regulations.

**Storage**
Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

**Specimen preparation**
Paraffin sections: The antibody can be used for labeling paraffin-embedded tissue sections fixed in formalin or Bouin's (3). Pre-treatment of deparaffinized tissues with proteinase K or heat-induced epitope retrieval is recommended. For heat-induced epitope retrieval of tissues fixed in formalin, optimal results are obtained with 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. Less optimal results are obtained with Dako Target Retrieval Solution, Code S1700, or 10 mmol/L citrate buffer, pH 6.0 The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure.

Frozen sections and cell preparations: The antibody can be used for labeling frozen sections (1, 3) and fixed cell smears (9). The user must validate the staining procedure.

Techninės specifikacijos 31 reikalavimas

**Staining procedure**
These are guidelines only. Optimal conditions may vary depending on specimen type and preparation method, and should be validated individually by each laboratory. The performance of this antibody should be established by the user when utilized with other manual staining systems or automated platforms.

Dilution: Monoclonal Mouse Anti-Human Cytokeratin 7, Code M7018, may be used at a dilution range of 1:50-1:100 when applied on formalin-fixed, paraffin-embedded sections of human breast and using 20 minutes heat-induced epitope retrieval in 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0and 30 minutes incubation at room temperature with the primary antibody. The recommended negative control is Dako Mouse IgG1, Code X0931, diluted to the same mouse IgG concentration as the primary antibody. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, it is recommended to dilute these reagents immediately before use, or dilute in Dako Antibody Diluent, Code S0809.

Quality control: Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimen.

Visualization: Dako EnVision+/HRP kits, e.g. Code K4005, are recommended. Follow the procedure enclosed with the selected visualization kit.

**Product-specific limitations**
Exceptions to the generally expected reactivity pattern may occur, for example, CK 7-labeled hepatocytes have been observed in patients with acute and chronic cholestasis (1).

Cells labeled by the antibody display a cytoplasmic staining pattern.

**Performance characteristics**
Normal tissues: The antibody consistently labels a large number of simple-, complex- and transitional epithelia, including biliary and pancreatic ducts, lung alveoli, endometrium, distal convoluted tubules and collecting ducts of the kidney (simple epithelia), bronchial and bronchiolar epithelium, ducts of prostate, luminal cells of fallopian tube and endocervix, bronchial-, breast-, salivary-, sweat- and endocervical glands, placental trophoblasts (complex epithelia), and all cell layers of urothelium (transitional epithelium) (3). Additionally, the antibody labels ovarian mesothelium (4), and labeling has also been observed in luminal- and basal cells of the prostate and myoepithelial cells (3). Further, in frozen sections, the antibody has been shown to label the rete epithelium in the testis, epididymis epithelium, and the surface epithelium of the stomach and duodenum (1). Non-epithelial tissues, such as connective tissue, blood vessels, and lymphoid tissue, are negative with the antibody (1).

Abnormal tissues: In the human ovary, 12/12 cystoma simplex, 12/12 cystadenomas and 60/60 carcinomas were labeled by the antibody (4). Furthermore, 6/6 endometrial, 4/4 endocervical-, 3/3 breast-, and 3/3 thyroid carcinomas were labeled (3). In frozen tissues of the female genital tract, 1/1 Brenner´s ovarian tumor, 21/21 serous- and 6/6 mucinous cystadenocarcinomas, 4/4 nonclassified ovarian carcinomas, 8/8 endometrioid carcinomas, 1/1 adenosquamous carcinoma of the cervix, 1/1 metastasis of fallopian tube adenocarcinoma in the cervix, and 1/1 placental choriocarcinoma revealed labeling with the antibody (1). In the human lung, 20/20 adenocarcinomas of different grades, including 4 bronchioalveolar carcinomas, were labeled with the antibody, whereas 24/24 cases of squamous cell carcinomas were unlabeled, as were 6/6 cases of large cell anaplastic carcinomas (2) and 10/10 cases of mesotheliomas (9). In the human gastrointestinal tract, 5/6 differently graded adenocarcinomas and 3/3 poorly differentiated signet ring cell carcinomas of the stomach, and 1/1 carcinoma of the pancreas were labeled by the antibody, whereas anaplastic stomach-, small intestine-, and rectal carcinomas as well as colon adenocarcinomas of different grades were unlabeled (3). In frozen tissues, 1/1 anaplastic adenocarcinoma of the stomach, 1/1 adenocarcinoma of jejunum, 2/2 intestinal carcinoids, 1/1 hepatoblastoma and 3/8 hepatocellular carcinomas were labeled. Also 4/4 transitional cell carcinomas were labeled (1). 6/6 chromophobe renal cell carcinomas were labeled, whereas 8/11 oncocytomas were unlabeled with the antibody (5).

## FRANÇAIS

**Utilisation prévue**
Pour utilisation diagnostique in vitro.

L'anticorps Monoclonal Mouse Anti-Human Cytokeratin 7, Clone OV-TL 12/30 est destiné à être utilisé en immunohistochimie (IHC). Cet anticorps marque les cellules épithéliales glandulaires et transitionnelles et facilite la classification des adénocarcinomes du poumon (1, 2), du sein et de l'endomètre, de la glande thyroïde (1, 3) et de l'ovaire (1, 4), ainsi que le carcinome (urothélial) à cellules transitionnelles (1) et le carcinome à cellules rénales chromophobes (5). La classification différentielle est facilitée par les résultats provenant d'un panel d'anticorps. L'interprétation clinique de toute coloration ou son absence doit être complétée par des études morphologiques en utilisant des contrôles appropriés et doit être évaluée en fonction des antécédents cliniques du patient et d'autres tests diagnostiques par un pathologiste qualifié. Cet anticorps est destiné à être utilisé après un diagnostic primaire de tumeur par histopathologie traditionnelle utilisant des colorations histochimiques non immunologiques.

**Résumé et explication**
La cytokératine 7 fait partie des filaments intermédiaires, qui créent un cytosquelette dans presque toutes les cellules eucaryotes. À la différence des autres filaments intermédiaires, les cytokératines (CK) sont constituées d'une famille multigénique hautement complexe de polypeptides dont les poids moléculaires varient entre 40 et 68 kDa. Les CK sont généralement perçues comme faisant partie des marqueurs les plus fondamentaux de la différenciation épithéliale, et jusqu'à présent, 20 polypeptides distincts de CK ont été mis en évidence dans différents épithéliums humains (6, 7). Les CK peuvent être divisées en sous-familles : type A acide (classe I) et type B neutre-basique (classe II). La CK7, une protéine de 54 kDa, appartient à la sous-famille de type B neutre-basique et sa répartition se limite aux épithéliums glandulaire et transitionnel (6).

Consulter le document *General Instructions for Immunohistochemical Staining* (Instructions générales de coloration immunohistochimique) de Dako ou les instructions du kit de détection pour les procédures IHC : Principe de la procédure, Matériel requis mais non fourni, Conservation, Préparation des échantillons, Procédure de coloration, Contrôle de qualité, Dépannage, Interprétation de la coloration, Limites générales.

**Réactif fourni**
Anticorps monoclonal de souris, fourni sous forme liquide en tant que surnageant de culture cellulaire, dialysé contre 0,05 mol/L de Tris-HCL à pH 7,2 et contenant 15 mmol/L d'azide de sodium ($NaN_3$).

Clone : OV-TL 12/30 (1). Isotype : IgG1, kappa.

Concentration en IgG de souris : Voir l'étiquette sur le flacon.

La concentration en protéines peut varier d'un lot à l'autre sans que cela influence la dilution optimale. Le titre de chaque lot est comparé et ajusté par rapport à un lot de référence pour assurer des performances de coloration immunohistochimiques cohérentes d'un lot à l'autre.

**Immunogène**
OTN 11, lignée cellulaire de carcinome ovarien (1, 8).

**Spécificité**
Dans les analyses PAGE en présence de SDS uni- ou bidimensionnelles et dans les immunoblots de préparations cytosquelettiques de plusieurs tissus et cultures cellulaires extraits par Triton X-100, l'anticorps marque une bande de 54 kDa correspondant à la cytokératine 7 (1).

**Précautions d'emploi**
1. Pour utilisation diagnostique in vitro.
2. Pour utilisateurs professionnels.
3. Ce produit contient de l'azide de sodium ($NaN_3$), un produit chimique hautement toxique à l'état pur. Aux concentrations du produit, bien que non classé comme dangereux, l'azide de sodium peut réagir avec le cuivre et le plomb des canalisations et former des accumulations d'azides métalliques hautement explosives. Lors de l'élimination, rincer abondamment à l'eau pour éviter toute accumulation d'azide métallique dans les canalisations.
4. Comme avec tout produit d'origine biologique, des procédures de manipulation appropriées doivent être respectées.
5. Porter un équipement de protection individuelle approprié pour éviter tout contact avec les yeux et la peau.
6. Les solutions non utilisées doivent être éliminées conformément aux réglementations locales, nationales et européennes.

**Conservation**
Conserver entre 2 et 8 °C. Ne pas utiliser après la date de péremption imprimée sur le flacon. Si les réactifs sont conservés dans des conditions autres que celles indiquées, celles-ci doivent être validées par l'utilisateur. Il n'existe pas de signe particulier pour indiquer l'instabilité de ce produit. Par conséquent, des contrôles positifs et négatifs doivent être testés en même temps que les échantillons de patient. Si une coloration inattendue est observée, qui ne peut être expliquée par des différences dans les procédures du laboratoire et qu'un problème lié à l'anticorps est suspecté, contacter l'assistance technique de Dako.

**Préparation des échantillons**
Coupes en paraffine : L'anticorps peut être utilisé pour le marquage des coupes de tissus incluses en paraffine et fixées au formol ou au liquide de Bouin (3). Le prétraitement des tissus déparaffinés par la protéinase K ou avec une restauration d'épitope induite par la chaleur est recommandé. Pour la restauration d'épitope induite par la chaleur des tissus fixés au formol, des résultats optimaux sont obtenus dans un tampon Tris à 10 mmol/L, EDTA à 1 mmol/L, à pH 9,0. Des résultats moins optimaux sont obtenus avec la solution Dako Target Retrieval Solution, réf. S1700, ou dans un tampon citrate à 10 mmol/L, à pH 6,0. Les coupes de tissu ne doivent pas sécher lors du traitement ni lors de la procédure de coloration immunohistochimique qui suit.

Coupes congelées et préparations cellulaires : L'anticorps peut être utilisé pour marquer des coupes congelées (1, 3) et des frottis cellulaires fixés (9). L'utilisateur doit valider la procédure de coloration.

**Procédure de coloration**
Il ne s'agit là que de conseils. Les conditions optimales peuvent varier en fonction du type de prélèvement et de la méthode de préparation, et doivent être validées individuellement par chaque laboratoire. Les performances de cet anticorps doivent être établies par l'utilisateur lorsqu'il est utilisé avec d'autres systèmes de coloration manuelle ou plates-formes automatisées.

Dilution : Le Monoclonal Mouse Anti-Human Cytokeratin 7, réf. M7018, peut être utilisé à une gamme de dilution de 1:50-1:100 lorsqu'il est appliqué sur des coupes de tissu mammaire humain fixées au formol et incluses en paraffine, en utilisant une restauration d'épitope induite par la chaleur de 20 minutes dans un tampon Tris à 10 mmol/L, EDTA à 1 mmol/L, à pH 9,0 et une incubation de 30 minutes avec l'anticorps primaire à température ambiante. Le contrôle négatif recommandé est le produit Dako Mouse IgG1, réf. X0931, dilué à la même concentration en IgG de souris que l'anticorps primaire. À moins que la stabilité de l'anticorps dilué et du contrôle négatif n'ait été établie dans la procédure de coloration en cours, il est recommandé de diluer ces réactifs immédiatement avant utilisation ou de les diluer avec le produit Dako Antibody Diluent, réf. S0809.

Contrôle de qualité : Les tissus de contrôle positifs et négatifs, ainsi que le réactif de contrôle négatif, doivent être testés en parallèle selon le même protocole que pour les échantillons de patients.

Visualisation : Il est recommandé d'utiliser les kits Dako EnVision+/HRP, réf. K4005. Suivre la procédure incluse dans le kit de visualisation sélectionnée.

**Limitations spécifiques du produit**
Des exceptions au schéma de réactivité généralement attendu sont observées : par exemple, les hépatocytes marqués par la CK 7 ont été observés chez des patients souffrant de cholestase aiguë et chronique (1).

Les cellules marquées par l'anticorps présentent un motif de coloration cytoplasmique.

**Performances**
Tissus sains : L'anticorps marque de manière homogène un grand nombre d'épithéliums simples, complexes et transitionnels, notamment les canaux biliaires et pancréatiques, les alvéoles pulmonaires, l'endomètre, les tubules contournés distaux et les tubes collecteurs du rein (épithéliums simples), l'épithélium bronchique et bronchiolaire, les canaux prostatiques, les cellules luminales des trompes de Fallope et de l'endocol, les glandes bronchiques, mammaires, salivaires, sudoripares et endocervicales, les trophoblastes placentaires (épithéliums complexes), ainsi que toutes les couches de l'urothélium (épithélium transitionnel) (3). En outre, l'anticorps marque le mésothélium ovarien (4) et un marquage a également été observé dans les cellules luminales et basales de la prostate et les cellules myoépithéliales (3). Par ailleurs, dans les coupes congelées, il a été montré que l'anticorps marque l'épithélium du rete testis, l'épithélium de l'épididyme et l'épithélium de surface de l'estomac et du duodénum (1). Les tissus non épithéliaux, tels le tissu conjonctif, les vaisseaux sanguins, et le tissu lymphoïde sont négatifs à l'anticorps (1).

Tissus anormaux : Dans l'ovaire humain : 12 kystomes simples sur 12, 12 cystadénomes sur 12 et 60 carcinomes sur 60 étaient marqués par l'anticorps (4). En outre, 6 carcinomes endométriaux sur 6, 4 carcinomes endocervicaux sur 4, 3 carcinomes mammaires sur 3 et 3 carcinomes thyroïdiens sur 3 étaient marqués (3). Dans les tissus congelés de l'appareil génital féminin, 1 tumeur de Brenner sur 1, 21 cystadénocarcinomes séreux sur 21 et 6 cystadénocarcinomes mucineux sur 6, 4 carcinomes ovariens non classés sur 4, 8 carcinomes endométrioïdes sur 8, 1 carcinome adénosquameux du col de l'utérus sur 1, 1 métastase de l'adénocarcinome des trompes de Fallope au niveau du col de l'utérus sur 1, et 1 choriocarcinome placentaire sur 1 étaient marqués par l'anticorps (1). Dans le poumon humain : 20 adénocarcinomes de différents grades sur 20, dont 4 carcinomes broncho-alvéolaires, étaient marqués par l'anticorps, alors que 24 carcinomes à cellules squameuses sur 24 n'étaient pas marqués, de même que 6 cas de carcinomes anaplasiques à grandes cellules sur 6 (2) et 10 cas de mésothéliomes sur 10 (9). Dans le tractus gastro-intestinal humain : 5 adénocarcinomes de grades différents sur 6, 3 carcinomes faiblement différenciés à cellules en bague de l'estomac sur 3 et 1 carcinome du pancréas sur 1 étaient marqués par l'anticorps, alors que les carcinomes anaplasiques de l'estomac, de l'intestin grêle et du rectum, ainsi que des adénocarcinomes du côlon de grades différents n'étaient par marqués (3). Dans les tissus congelés, 1 adénocarcinome anaplasique de l'estomac sur 1, 1 adénocarcinome du jéjunum sur 1, 2 carcinoïdes intestinaux sur 2, 1 hépatoblastome sur 1 et 3 carcinomes hépatocellulaires sur 8 étaient marqués. Par ailleurs, 4 carcinomes à cellules transitionnelles sur 4 étaient marqués (1). 6 carcinomes rénaux à cellules chromophobes sur 6 étaient marqués, alors que 8 oncocytomes sur 11 n'étaient pas marqués par l'anticorps (5).

## DEUTSCH

**Verwendungszweck**
Zur In-vitro-Diagnostik.

Monoclonal Mouse Anti-Human Cytokeratin 7, Clone OV-TL 12/30 ist für die Verwendung in der Immunhistochemie (IHC) bestimmt. Dieser Antikörper markiert Drüsen- und Übergangsepithelzellen und unterstützt die Klassifizierung von Adenokarzinomen von Lunge (1, 2), Brust und Endometrium, Schilddrüse (1, 3) und Eierstöcken (1, 4) sowie von Übergangsepithelkarzinomen (Urothelialkarzinomen) (1) und chromophoben Nierenzellkarzinomen (5). Die Differenzialklassifikation wird durch die Ergebnisse eines Antikörper-Panels unterstützt. Die klinische Auswertung einer eintretenden oder ausbleibenden Färbung sollte durch morphologische Studien mit geeigneten Kontrollen ergänzt werden und von einem qualifizierten Pathologen unter Berücksichtigung der Krankengeschichte und anderer diagnostischer Tests des Patienten vorgenommen werden. Dieser Antikörper kommt nach der Primärdiagnose des Tumors durch konventionelle Histopathologie unter Verwendung von nicht immunologischen histochemischen Färbungen zum Einsatz.

**Zusammenfassung und Erklärung**
Cytokeratin 7 gehört zu den Intermediärfilamenten, die in fast allen eukaryotischen Zellen ein Zytoskelett bilden. Im Gegensatz zu anderen Intermediärfilamentproteinen bilden Zytokeratine (ZK) eine hochkomplexe, von einer Multigenfamilie codierte Familie von Polypeptiden, deren Molekulargewicht von 40 bis 68 kDa reicht. ZK werden zu den wichtigsten Markern für epitheliale Differenzierung gezählt. Bisher wurden 20 unterschiedliche ZK-Polypeptide in verschiedenen menschlichen Epithelien entdeckt (6, 7). Die ZK lassen sich in einen sauren Typ A (Klasse I) und einen neutral-basischen Typ B (Klasse II) unterteilen. Das 54-kDa-Protein CK7 gehört zur neutral-basischen Unterfamilie Typ B, und seine Verteilung ist auf Drüsen- und Übergangsepithelien begrenzt (6).

Folgende Angaben bitte den *General Instructions for Immunohistochemical Staining* (Allgemeine Richtlinien zur immunhistochemischen Färbung) von Dako bzw. den Anweisungen des Detektionssystems für IHC-Verfahren entnehmen: Verfahrensprinzipien, Erforderliche, aber nicht mitgelieferte Materialien, Lagerung, Gewebevorbereitung, Färbeverfahren, Qualitätskontrolle, Fehlerbehandlung, Auswertung der Färbung, Allgemeine Beschränkungen.

**Geliefertes Reagenz**
Monoklonale Mausantikörper in flüssiger Form als gegen 0.05 mol/L Tris-HCl, pH 7.2 und 15 mmol/L $NaN_3$ dialysierter Zellkulturüberstand.

Klon: OV-TL 12/30 (1). Isotyp: IgG1, Kappa.

Konzentration von Maus-IgG: Siehe Behälteretikett.

Die Proteinkonzentration kann zwischen Chargen abweichen, ohne die optimale Verdünnung zu beeinflussen. Der Titer jeder Charge wird mit dem einer Referenzcharge verglichen und dieser angeglichen, um konstante immunhistochemische Färbeergebnisse zwischen den Chargen zu gewährleisten.

**Immunogen**
OTN 11, Eierstockkarzinom-Zelllinie (1, 8).

**Spezifität**
Beim ein- und zweidimensionalen SDS-PAGE und Immunblotting der Triton X-100-extrahierten Zellskelettpräparate mehrerer Zellkulturen und Gewebe markierte der Antikörper eine Cytokeratin 7 entsprechende Bande von 54 kDa (1).

**Vorsichtsmaßnahmen**
1. Zur In-vitro-Diagnostik.
2. Für Fachpersonal.
3. Dieses Produkt enthält Natriumazid ($NaN_3$), eine in reiner Form äußerst giftige Chemikalie. Bei den in diesem Produkt verwendeten Konzentrationen kann Natriumazid, obwohl nicht als gefährlich klassifiziert, mit in Wasserleitungen vorhandenem Blei oder Kupfer reagieren und zur Bildung von hochexplosiven Metallazid-Anreicherungen führen. Nach der Entsorgung muss mit reichlich Wasser nachgespült werden, um Metall-Azid-Anreicherung zu vermeiden.
4. Wie alle Produkte biologischen Ursprungs müssen auch diese entsprechend gehandhabt werden.
5. Geeignete persönliche Schutzausrüstung (PSA) tragen, um Augen- und Hautkontakt zu vermeiden.
6. Nicht verwendete Lösung ist entsprechend örtlichen, staatlichen und EU-rechtlichen Richtlinien zu entsorgen.

**Lagerung**
Bei 2-8 °C lagern. Nach Ablauf des auf dem Behälter aufgedruckten Verfallsdatums nicht mehr verwenden. Werden die Reagenzien unter anderen als den angegebenen Bedingungen aufbewahrt, müssen diese Bedingungen vom Benutzer überprüft werden. Es gibt keine offensichtlichen Anhaltspunkte für die mögliche Instabilität dieses Produkts. Es sollten daher die Positiv- und Negativkontrollen gleichzeitig mit den Patientengewebeproben mitgeführt werden. Wenn eine unerwartete Anfärbung beobachtet wird, welche durch Änderungen in den Labormethoden nicht erklärt werden kann, und falls Verdacht auf ein Problem mit dem Antikörper besteht, ist Kontakt mit dem technischen Kundendienst von Dako aufzunehmen.

**Gewebevorbereitung**
Paraffinschnitte: Der Antikörper kann für die Markierung von paraffineingebetteten, in Formalin oder Bouin-Lösung (3) fixierten Gewebeschnitten verwendet werden. Die Vorbehandlung entparaffinierter Gewebe durch Proteinase K oder hitzeinduzierte Epitopdemaskierung wird empfohlen. Bei der hitzeinduzierten Epitopdemaskierung formalinfixierter Gewebe werden optimale Resultate mit 10 mmol/L Tris-Puffer, 1 mmol/L EDTA, pH 9.0 erzielt. Weniger optimale Resultate werden mit Dako Target Retrieval

Solution, Code-Nr. S1700, oder 10 mmol/L Zitratpuffer, pH 6.0, erzielt. Die Gewebeschnitte dürfen während der Behandlung oder des anschließenden immunhistochemischen Färbeverfahrens nicht austrocknen.

Gefrierschnitte und Zellpräparate: Der Antikörper eignet sich zur Markierung von Gefrierschnitten (1, 3) und fixierten Zellausstrichen (9). Das Färbeverfahren muss vom Anwender validiert werden.

**Färbeverfahren**
Diese Angaben sind nur Richtlinien. Optimale Bedingungen können je nach Gewebetyp und Vorbereitungsverfahren unterschiedlich sein und sollten vom jeweiligen Labor selbst ermittelt werden. Die Leistung dieses Antikörpers sollte vom Benutzer bei einem Einsatz mit anderen manuellen Färbesystemen oder automatisierten Systemen ermittelt werden.

Verdünnung: Monoclonal Mouse Anti-Human Cytokeratin 7, Code-Nr. M7018, kann auf formalinfixierten, paraffineingebetteten Schnitten von humanem Brustgewebe bei einer hitzeinduzierten Epitopdemaskierung von 20 Minuten in 10 mmol/L Tris-Puffer, 1 mmol/L EDTA, pH 9.0, und einer 30-minütigen Inkubation mit dem primären Antikörper bei Raumtemperatur in einem Verdünnungsbereich zwischen 1:50 und 1:100 verwendet werden. Als Negativkontrolle wird Dako Mouse IgG1, Code-Nr. X0931, empfohlen, das auf dieselbe Konzentration an Maus-IgG wie der primäre Antikörper verdünnt wurde. Falls die Stabilität des verdünnten Antikörpers und der Negativkontrolle für das verwendete Färbeverfahren nicht erwiesen ist, wird empfohlen, diese Reagenzien unmittelbar vor der Verwendung bzw. in Dako Antibody Diluent, Code-Nr. S0809, zu verdünnen.

Qualitätskontrolle: Positiv- und Negativkontrollgewebe sowie Negativkontrollreagenz sollten zur gleichen Zeit und mit demselben Protokoll wie die Patientengewebe getestet werden.

Detektionssystem: Dako EnVision+/HRP Kits (z. B. Code-Nr. K4005) werden empfohlen. Das für das ausgewählte Detektionssystem beschriebene Verfahren befolgen.

**Produktspezifische Beschränkungen**
Ausnahmen des allgemein erwarteten Reaktivitätsmuster sind möglich. So wurden beispielsweise CK 7-markierte Hepatozyten bei Patienten mit akuter und chronischer Cholestase beobachtet (1).

Mit diesem Antikörper markierte Zellen weisen ein zytoplasmatisches Färbemuster auf.

**Leistungseigenschaften**
Normale Gewebe: Der Antikörper markiert konsistent eine große Anzahl einfacher, komplexer und Übergangsepithelien, u. a.: Gallen- und Pankreasgänge, Alveolen der Lunge, Endometrium, distale geknäulte Anteile (Partes convolutae) der Tubuli und Sammelgänge der Niere (einfache Epithelien), bronchiales und bronchiolares Epithel, Prostatagänge, luminale Zellen von Salpinx und Endozervix, Drüsen der Bronchien und Brust, Speichel- und Schweißdrüsen, endozervikale Drüsen, Trophoblasten der Plazenta (komplexe Epithelien) und sämtliche Zellschichten des Urothels (Übergangsepithel) (3). Zudem markiert der Antikörper das Eierstock-Mesothel (4). Ebenso wurde eine Markierung bei Luminal- und Basalzellen der Prostata und bei Myoepithelzellen beobachtet (3). Es wurde zudem nachgewiesen, dass der Antikörper in Gefrierschnitten das Netzepithel der Hoden, das Epididymisepithel sowie das Oberflächenepithele des Magens und des Duodenums markiert (1). Epithelfreie Gewebe wie Bindegewebe, Blutgefäße und lymphoides Gewebe reagieren negativ auf den Antikörper (1).

Anormale Gewebe: Im menschlichen Eierstock markierte der Antikörper 12 von 12 Cystoma simplex, 12 von 12 Cystadenomen und 60 von 60 Karzinomen (4). Zudem wurden 6 von 6 Endometrium-, 4 von 4 Endozervikal-, 3 von 3 Brust- und 3 von 3 Schilddrüsenkarzinomen markiert (3). In Gefrierschnitten aus dem weiblichen Genitaltrakt wurden 1 von 1 Brennertumor, 21 von 21 serösen und 6 von 6 mukösen Zystadenokarzinomen, 4 von 4 nichtklassifizierten Ovarialkarzinomen, 8 von 8 endometrioiden Karzinomen, 1 von 1 adenosquamösen Brustkarzinom, 1 von 1 Brustmetastase eines Adenokarzinoms des Eileiters und 1 von 1 plazentaren Choriokarzinom mit dem Antikörper markiert (1). Bei der menschlichen Lunge wurden 20 von 20 Adenokarzinomen unterschiedlicher Malignität, einschließlich 4 bronchioalveolarer Karzinome, durch den Antikörper markiert. Dagegen wurden 24 von 24 Fällen von Plattenepithelkarzinomen nicht markiert, ebenso wie 6 von 6 Fällen großzelliger anaplastischer Karzinome (2) und 10 von 10 Fällen von Mesotheliomen (9). Beim menschlichen Gastrointestinaltrakt wurden 5 von 6 Adenokarzinomen unterschiedlicher Malignität und 3 von 3 schlecht differenzierten Siegelringzellkarznomen des Magens und 1 von 1 Pankreaskarzinom durch den Antikörper markiert. Dagegen wurden anaplastische Adenokarzinome des Magens, Dünndarms und Rektums sowie Adenokarzinome des Dickdarms mit unterschiedlicher Malignität nicht markiert (3). In Gefrierschnitten wurden 1 von 1 anaplastischen Adenokarzinom des Magens, 1 von 1 Adenokarzinom des Jejunums, 2 von 2 intestinalen Karzioniden, 1 von 1 Hepatoblastom und 3 von 8 hepatozellulären Karzinomen markiert. Auch 4 von 4 Übergangsepithelkarzinomen wurden markiert (1). 6 von 6 chromophoben Nierenzellenkarzinomen wurden markiert, während 8 von 11 Onkozytomen nicht durch den Antikörper markiert wurden (5).

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**Explanation of symbols / Explication des symboles / Erläuterung der Symbole**

| | | |
|---|---|---|
| REF<br>Catalogue number<br>Référence du catalogue<br>Katalognummer | 2 °C – 8 °C<br>Temperature limitation<br>Limites de température<br>Zulässiger Temperaturbereich | Use by<br>Utiliser avant<br>Verwendbar bis |
| IVD<br>In vitro diagnostic medical device<br>Dispositif médical de diagnostic in vitro<br>In-vitro-Diagnostikum | LOT<br>Batch code<br>Réf. du lot<br>Chargenbezeichnung | Manufacturer<br>Fabricant<br>Hersteller |
| Consult instructions for use<br>Consulter les instructions d'utilisation<br>Gebrauchsanweisung beachten | EC REP<br>Authorized representative in the European Community<br>Représentant agréé dans la Communauté européenne<br>Autorisierte Vertretung in der Europäischen Gemeinschaft | |


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Revision / Révision / Revision 2020.11

**Monoclonal Mouse Anti-Human Cytokeratin 20**
Clone $K_s$ 20.8
**Code M7019**

Technines specifikacijos 30 reikalavimas

## ENGLISH

**Intended use**

For in vitro diagnostic use.

Monoclonal Mouse Anti-Human Cytokeratin 20, Clone $K_s$ 20.8, is intended for use in immunohistochemistry (IHC). This antibody labels normal and abnormal gastric and intestinal epithelium, urothelium and Merkel cells (1, 2), and the antibody is a useful aid in the classification of carcinomas originating from these cell types (1, 3, 4, 5). Differential classification is aided by the results from a panel of antibodies. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

**Synonym for antigen**

Protein IT (6).

**Summary and explanation**

Cytokeratin 20 (CK 20) belongs to the intermediate filament proteins, which create a cytoskeleton in almost all cells. In contrast to other intermediate filaments, cytokeratins (CKs) are made up of a highly complex multigene family of 40 to 68 kDa polypeptides (1, 7). 20 distinct CK polypeptides have been revealed in various human epithelial cells and their malignant counterparts (1, 2, 7). They can be divided into an acidic (type I) and a neutral-basic (type II) subfamily. CK 20, a 46 kDa protein, is less acidic than the other type I cytokeratins. CK 20 labeling (5-100 % labeled tumor cells) has been reported in the vast majority of adenocarcinomas of the colon, mucinous ovarian tumors, transitional-cell and Merkel cell carcinomas, and frequently also in adenocarcinomas of the stomach, bile system, and pancreas (40%). Very few breast and lung adenocarcinomas and squamous cell carcinomas were labeled, while adenocarcinomas of the ovary (non-mucinous) and endometrium, and renal cell and small cell carcinomas showed only scattered labeled cells in some cases (1).

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure; Materials Required, Not Supplied; Storage; Specimen Preparation; Staining Procedure; Quality Control; Troubleshooting; Interpretation of Staining; General Limitations.

**Reagent provided**

Monoclonal mouse antibody provided in liquid form as purified mouse IgG from ascitic fluid. In 0.05 mol/L Tris-HCL, 15 mmol/L $NaN_3$, 1 % bovine serum albumin, pH 7.2.

Clone: $K_s$ 20.8 (1). Isotype: IgG2a, kappa.

Mouse IgG concentration: see label on vial.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

**Immunogen**

CK 20 isolated by SDS-PAGE from cytoskeletal material obtained from human duodenal mucosa (1).

**Specificity**

Anti-Human CK 20, Clone $K_s$ 20.8, labels a 46 kDa polypeptide corresponding to CK 20 in Western immunoblotting of SDS-PAGE-separated cytoskeletal proteins from human duodenal mucosa (1).

**Precautions**

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution should be disposed of according to local, State and Federal regulations.

**Storage**

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

**Specimen preparation**

Paraffin sections: The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections fixed in formalin. Pre-treatment of deparaffinized tissues with trypsin (4), proteinase K or heat-induced epitope retrieval is required. For heat-induced epitope retrieval, optimal results are obtained with Dako Target Retrieval Solution, Code S1700, or 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. Less optimal results are obtained with 10 mmol/L citrate buffer, pH 6.0. The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure.

Frozen sections and cell preparations: The antibody cannot be used for labeling frozen sections or cell smears owing to cross-reactivity with some cytokeratin-20-negative epithelia (e.g. renal tubules) (1). The user must validate the staining procedure.

**Staining procedure**

These are guidelines only. Optimal conditions may vary depending on specimen type and preparation method, and should be validated individually by each laboratory. The performance of this antibody should be established by the user when utilized with other manual staining systems or automated platforms.

Technines specifikacijos 30 reikalavimas

Dilution: Monoclonal Mouse Anti-Human Cytokeratin 20, Code M7019, may be used at a dilution range of 1:25-1:50 when applied on formalin-fixed, paraffin-embedded sections of human colon, and using 20 minutes heat-induced epitope retrieval in Dako Target Retrieval Solution, Code S1700, and 30 minutes incubation at room temperature with the primary antibody. The recommended negative control is Dako Mouse IgG2a, Code X0943, diluted to the same mouse IgG concentration as the primary antibody. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, it is recommended to dilute these reagents immediately before use, or dilute in Dako Antibody Diluent, Code S0809.

Quality control: Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens.

Visualization: Dako EnVision+/HRP kits, e.g. Code K4005, are recommended. Follow the procedure enclosed with the selected visualization kit.

**Product-specific limitations**

CK 20 may occasionally be expressed in breast and lung adenocarcinomas, and in squamous cell carcinomas (1, 3). Less than 5% CK-20 labeled cells may be present in a number of tissues (1).

**Staining interpretation**

Cells labeled by the antibody display a cytoplasmic staining pattern.

**Performance characteristics**

Normal tissues: The antibody labels normal urothelium (1, 4) and the mature epithelium lining the villi of duodenal mucosa (1). CK 20 immunoreactivity has not been detected in a number of non-epithelial tissues tested, such as smooth muscle, blood vessel walls, lymph nodes and tumor stroma (1).

Abnormal tissues: In colonic adenocarcinoma, the antibody labeled 26 of 27 tumors (96%). 21 (78%) displayed staining in more than 50% of the cells (3). Of 51 patients with urothelial papillomas of the bladder, 10 showed labeling of superficial urothelial cells (normal labeling) with the antibody. By contrast, 30 patients (73%) showed CK 20 expression in all cell layers (abnormal labeling) (4). 3 out of 4 metastatic gastric tumors were labeled by the antibody (5).

Focal immunostaining with the antibody (less than 10% labeled cells) was observed in 9 of 65 lung, 1 of 20 breast, and 2 of 11 endometrial tumors. 1 of 19 clear-cell type renal cell carcinomas was labeled in 10-50% of the cells (3). None of 10 primary ovarian carcinomas, and none of 5 mesotheliomas were found labeled by the antibody (5).

## FRANÇAIS

**Utilisation prévue**

Pour utilisation diagnostique in vitro.

L'anticorps Monoclonal Mouse Anti-Human Cytokeratin 20, Clone $K_s$20.8 est destiné à être utilisé en immunohistochimie (IHC). Cet anticorps marque l'épithélium gastrique et intestinal sain et anormal, l'urothélium et les cellules de Merkel (1, 2) et facilite la classification des carcinomes issus de ces types de cellules (1, 3, 4, 5). La classification différentielle est facilitée par les résultats provenant d'un panel d'anticorps. L'interprétation clinique de toute coloration ou son absence doit être complétée par des études morphologiques en utilisant des contrôles appropriés et doit être évaluée en fonction des antécédents cliniques du patient et d'autres tests diagnostiques par un pathologiste qualifié. Cet anticorps est destiné à être utilisé après un diagnostic primaire de tumeur par histopathologie traditionnelle utilisant des colorations histochimiques non immunologiques.

**Synonyme de l'antigène**

Protéine IT (6).

**Résumé et explication**

La cytokératine 20 (CK 20) fait partie des protéines de filaments intermédiaires qui créent un cytosquelette dans presque toutes les cellules. À la différence des autres filaments intermédiaires, les cytokératines (CK) sont constituées d'une famille multigénique hautement complexe de polypeptides de 40 à 68 kDa (1, 7). On a recensé 20 CK différentes dans diverses cellules épithéliales humaines et leurs homologues tumorales (1, 2, 7). Les CK peuvent être divisées en sous-familles : type I acide et type II neutre-basique. La CK 20, une protéine de 46 kDa, est moins acide que les autres cytokératines de type I. La CK 20 a été marquée (5 à 100% de cellules tumorales marquées) dans la majeure partie des adénocarcinomes du côlon, des tumeurs ovariennes mucineuses, des carcinomes à cellules transitionnelles et à cellules de Merkel et également souvent dans les adénocarcinomes de l'estomac, du système biliaire et du pancréas (40%). Très peu d'adénocarcinomes du sein et du poumon et de carcinomes à cellules squameuses ont été marqués, alors que les adénocarcinomes de l'ovaire (non mucineux) et de l'endomètre, ainsi que les carcinomes à cellules rénales et à petites cellules ont montré uniquement des cellules marquées isolées dans certains cas (1).

Consulter le document *General Instructions for Immunohistochemical Staining* (Instructions générales de coloration immunohistochimique) de Dako ou les instructions du système de détection pour les procédures IHC : Principe de la procédure, Matériel requis mais non fourni, Conservation, Préparation des échantillons, Procédure de coloration, Contrôle de qualité, Dépannage, Interprétation de la coloration, Limites générales.

**Réactif fourni**

Anticorps monoclonal de souris fourni sous forme liquide comme IgG purifiée de souris provenant du liquide d'ascite. Dans un tampon Tris-HCL à 0,05 mol/L, 15 mmol/L de $NaN_3$ et 1% d'albumine de sérum bovin, à pH 7,2.

Clone : $K_s$ 20.8 (1). Isotype : IgG2a, kappa.

Concentration en IgG de souris : Voir l'étiquette sur le flacon.

La concentration en protéines peut varier d'un lot à l'autre sans que cela influence la dilution optimale. Le titre de chaque lot est comparé et ajusté par rapport à un lot de référence pour assurer des performances de coloration immunohistochimiques cohérentes d'un lot à l'autre.

**Immunogène**

CK 20 isolée par SDS-PAGE de matériau cytosquelettique obtenu à partir de muqueuse duodénale humaine (1).

**Spécificité**

L'anticorps Anti-Human CK 20, Clone $K_s$ 20.8 marque un polypeptide de 46 kDa correspondant à la CK 20 dans les analyses par Western immunoblot de protéines cytosquelettiques séparées par SDS-PAGE provenant de la muqueuse duodénale humaine (1).

**Précautions d'emploi**

1. Pour utilisation diagnostique in vitro.

2. Pour utilisateurs professionnels.

3. Ce produit contient de l'azide de sodium ($NaN_3$), produit chimique hautement toxique dans sa forme pure. Aux concentrations du produit, bien que non classé comme dangereux, l'azide de sodium peut réagir avec le cuivre et le plomb des canalisations et former des accumulations d'azides métalliques hautement explosives. Lors de l'élimination, rincer abondamment à l'eau pour éviter toute accumulation d'azide métallique dans les canalisations.

4. Comme avec tout produit d'origine biologique, respecter les procédures de manipulation appropriées.

5. Porter un équipement de protection individuelle approprié pour éviter tout contact avec les yeux et la peau.

6. Les solutions non utilisées doivent être éliminées conformément aux réglementations locales, nationales et européennes.

**Conservation**

Conserver entre 2 et 8 °C. Ne pas utiliser après la date de péremption imprimée sur le flacon. Si les réactifs sont conservés dans des conditions autres que celles indiquées, celles-ci doivent être validées par l'utilisateur. Il n'existe pas de signe particulier pour indiquer l'instabilité de ce produit. Par conséquent, des contrôles positifs et négatifs doivent être testés en même temps que les échantillons de patient. Si une coloration inattendue est observée, qui ne peut être expliquée par des différences dans les procédures du laboratoire et qu'un problème lié à l'anticorps est suspecté, contacter l'assistance technique de Dako.

**Préparation des échantillons**

Coupes en paraffine : L'anticorps peut être utilisé pour le marquage des coupes de tissus incluses en paraffine et fixées au formol. Le prétraitement des tissus déparaffinés par la trypsine (4), la protéinase K ou avec une restauration d'épitope induite par la chaleur (HIER) est nécessaire. Pour la restauration d'épitope induite par la chaleur, des résultats optimaux sont obtenus avec la solution Dako Target Retrieval Solution, réf. S1700 ou avec un tampon Tris à 10 mmol/L, EDTA à 1 mmol/L, à pH 9,0. Des résultats moins optimaux sont obtenus dans un tampon citrate à 10 mmol/L, à pH 6,0. Les coupes de tissus ne doivent pas sécher lors du traitement ni lors de la procédure de coloration immunohistochimique suivante.

Coupes congelées et préparations cellulaires : L'anticorps ne peut pas être utilisé pour le marquage de coupes congelées ou de frottis cellulaires en raison de la réactivité croisée avec certains épithéliums négatifs à la cytokératine 20 (par ex. les tubules rénaux) (1). L'utilisateur doit valider la procédure de coloration.

**Procédure de coloration**

Il ne s'agit là que de conseils. Les conditions optimales peuvent varier en fonction du type de prélèvement et de la méthode de préparation, et doivent être validées individuellement par chaque laboratoire. Les performances de cet anticorps doivent être établies par l'utilisateur lorsqu'il est utilisé avec d'autres systèmes de coloration manuelle ou plates-formes automatisées.

Dilution : L'anticorps Monoclonal Mouse Anti-Human Cytokeratin 20, réf. M7019 peut être utilisé à une gamme de dilution de 1:25 à 1:50 lorsqu'il est appliqué sur des coupes de côlon humain fixées au formol et incluses en paraffine, en utilisant une restauration d'épitope induite par la chaleur de 20 minutes dans la solution Dako Target Retrieval Solution, réf. S1700, et une incubation de 30 minutes avec l'anticorps primaire à température ambiante. Le contrôle négatif recommandé est le produit Dako Mouse IgG2a, réf. X0943, dilué à la même concentration en IgG de souris que l'anticorps primaire. À moins que la stabilité de l'anticorps dilué et du contrôle négatif n'ait été établie dans la procédure de coloration en cours, il est recommandé de diluer ces réactifs immédiatement avant utilisation ou de les diluer avec le produit Dako Antibody Diluent, réf. S0809.

Contrôle de qualité : Les tissus de contrôle positifs et négatifs, ainsi que le réactif de contrôle négatif, doivent être testés en parallèle selon le même protocole que pour les échantillons de patients.

Visualisation : Il est recommandé d'utiliser les kits Dako EnVision+/HRP, réf. K4005. Suivre la procédure incluse dans le kit de visualisation sélectionnée.

**Limitations spécifiques du produit**

La CK 20 peut occasionnellement être exprimée dans les adénocarcinomes du sein et du poumon et dans les carcinomes à cellules squameuses (1, 3). Moins de 5% de cellules marquées à la CK 20 peuvent être présentes dans différents tissus (1).

**Interprétation de la coloration**

Les cellules marquées par l'anticorps présentent un motif de coloration cytoplasmique.

**Performances**

Tissus sains : L'anticorps marque l'urothélium sain (1, 4) et l'épithélium mature qui tapisse les villosités de la muqueuse duodénale (1). Aucune immunoréactivité à la CK 20 n'a été détectée dans plusieurs tissus non épithéliaux testés (muscle lisse, paroi de vaisseau sanguin, ganglion lymphatique et stroma tumoral) (1).

Tissus anormaux : Dans l'adénocarcinome du côlon, l'anticorps a marqué 26 tumeurs sur 27 (96%). 21 (78%) de ces tumeurs présentaient une coloration de plus de 50% des cellules (3). Sur 51 patients atteints d'un papillome urothélial de la vessie, 10 ont présenté un marquage des cellules urothéliales superficielles (coloration normale) avec l'anticorps. En revanche, 30 patients (73%) présentaient une expression de la CK 20 dans toutes les couches cellulaires (coloration anormale) (4). Trois tumeurs gastriques métastatiques sur 4 ont été marquées par l'anticorps (5).

Une coloration immunologique focale avec l'anticorps (moins de 10% de cellules marquées) a été observée sur 9 tumeurs du poumon sur 65, 1 tumeur du sein sur 20 et 2 tumeurs de l'endomètre sur 11. Un carcinome rénal à cellules claires sur 19 a été marqué pour 10 à 50% des cellules (3). Aucun carcinome ovarien primitif sur 10 et aucun mésothéliome sur 5 n'a été marqué avec l'anticorps (5).

## DEUTSCH

**Verwendungszweck**

Zur In-vitro-Diagnostik.

Monoclonal Mouse Anti-Human Cytokeratin 20, Clone $K_s$ 20.8 ist zur Verwendung in der Immunhistochemie (IHC) bestimmt. Dieser Antikörper markiert normale und anormale gastrointestinale Epithel-, Urothel- und Merkel-Zellen (1, 2). Der Antikörper unterstützt die Klassifizierung von Karzinomen, die von diesen Zelltypen (1, 3, 4, 5) verursacht werden. Die Differenzialklassifikation wird durch die Ergebnisse eines Antikörper-Panels unterstützt. Die klinische Auswertung einer eintretenden oder ausbleibenden Färbung sollte durch morphologische Studien mit geeigneten Kontrollen ergänzt werden und von einem qualifizierten Pathologen unter Berücksichtigung der Krankengeschichte und anderer diagnostischer Tests des Patienten vorgenommen werden. Dieser Antikörper kommt nach der Primärdiagnose des Tumors durch konventionelle Histopathologie unter Verwendung von nicht immunologischen histochemischen Färbungen zum Einsatz.

**Synonym für das Antigen**

Protein IT (6).

**Zusammenfassung und Erklärung**

Cytokeratin 20 (CK 20) gehört zu den Intermediärfilamentproteien, die in fast allen Zellen ein Zytoskelett bilden. Im Gegensatz zu anderen intermediären Filamentproteinen bestehen Zytokeratine (ZK) aus einer hochkomplexen Multigenfamilie aus 40 bis 68 kDa-Polypeptiden (1, 7). Bisher wurden 20 unterschiedliche ZK-Polypeptide in verschiedenen menschlichen Epithelzellen und deren malignen Entsprechungen (1, 2, 7) nachgewiesen. Die ZK lassen sich in einen sauren Typ A (Klasse I) und einen neutral-basischen Typ B (Klasse II) unterteilen. CK 20, ein 46-kDa-Protein, ist weniger sauer als die Zytokeratine vom Typ I. In der überwiegenden Zahl von Adenokarzinomen des Dickdarms, muzinösen Eierstock-Tumoren, Karzinomen der Übergangsepithel- und Merkel-Zellen sowie häufig auch in Adenokarzinomen des Magens, des Gallensystems und des Pankreas (40%) wurden CK-20-Markierungen ermittelt (5-100% CK-20-markierte Tumorzellen). Sehr wenige Adenokarzinome der Brust und der Lunge sowie Plattenepithelkarzinome wurden markiert, wogegen Adenokarzinome der Eierstöcke (nicht muzinös) und der Uterusschleimhaut sowie Nierenzell- und kleinzellige Karzinome nur in wenigen Fällen vereinzelt markiert wurden (1).

Folgende Angaben bitte den *General Instructions for Immunohistochemical Staining* (Allgemeine Richtlinien zur immunhistochemischen Färbung) von Dako bzw. den Anweisungen des Detektionssystems für IHC-Verfahren entnehmen: Verfahrensprinzipien, Erforderliche, aber nicht mitgelieferte Materialien, Lagerung, Gewebevorbereitung, Färbeverfahren, Qualitätskontrolle, Fehlerbehandlung, Auswertung der Färbung, Allgemeine Beschränkungen.

**Geliefertes Reagenz**

Monoklonale Maus-Antikörper in flüssiger Form als gereinigte Maus-IgG aus Aszitesflüssigkeit. In 0.05 mol/L Tris-HCL, 15 mmol/L $NaN_3$, 1% Rinderserum-Albumin, pH 7.2.

Klon: $K_s$ 20.8 (1). Isotyp: IgG2a, Kappa.

Konzentration von Maus-IgG: Siehe Behälteretikett.

Die Proteinkonzentration kann zwischen Chargen abweichen, ohne die optimale Verdünnung zu beeinflussen. Der Titer jeder Charge wird mit dem einer Referenzcharge verglichen und dieser angeglichen, um konstante immunhistochemische Färbeergebnisse zwischen den Chargen zu gewährleisten.

**Immunogen**

Mit SDS-PAGE aus Zytoskelettmaterial, das aus menschlicher Duodenalmukosa (1) gewonnen wurde, isoliertes CK 20.

**Spezifität**

Anti-Human CK 20, Clone $K_s$ 20.8, markiert ein 46-kDa-Polypeptid entsprechend CK 20 beim Western-Immunblotting von durch SDS-PAGE abgeschiedenen Zytoskelettproteinen aus der menschlichen Duodenalmukosa (1).

**Vorsichtsmaßnahmen**

1. Zur In-vitro-Diagnostik.
2. Für Fachpersonal.
3. Dieses Produkt enthält Natriumazid ($NaN_3$), eine in reiner Form äußerst giftige Chemikalie. Bei den in diesem Produkt verwendeten Konzentrationen kann Natriumazid, obwohl nicht als gefährlich klassifiziert, mit in Wasserleitungen vorhandenem Blei oder Kupfer reagieren und zur Bildung von hochexplosiven Metallazid-Anreicherungen führen. Nach der Entsorgung muss mit reichlich Wasser nachgespült werden, um Metall-Azid-Anreicherung zu vermeiden.
4. Wie alle Produkte biologischen Ursprungs müssen auch diese entsprechend gehandhabt werden.
5. Geeignete persönliche Schutzausrüstung (PSA) tragen, um Augen- und Hautkontakt zu vermeiden.
6. Nicht verwendete Lösung ist entsprechend örtlichen, staatlichen und EU-rechtlichen Richtlinien zu entsorgen.

**Lagerung**

Bei 2-8 °C lagern. Nach Ablauf des auf dem Behälter aufgedruckten Verfallsdatums nicht mehr verwenden. Werden die Reagenzien unter anderen als den angegebenen Bedingungen aufbewahrt, müssen diese Bedingungen vom Benutzer überprüft werden. Es gibt keine offensichtlichen Anhaltspunkte für die mögliche Instabilität dieses Produkts. Es sollten daher die Positiv- und Negativkontrollen gleichzeitig mit den Patientengewebeproben mitgeführt werden. Wenn eine unerwartete Anfärbung beobachtet wird, welche durch Änderungen in den

Labormethoden nicht erklärt werden kann, und falls Verdacht auf ein Problem mit dem Antikörper besteht, ist Kontakt mit dem technischen Kundendienst von Dako aufzunehmen.

**Gewebevorbereitung**

Paraffinschnitte: Der Antikörper kann für die Markierung von formalinfixierten, paraffineingebetteten Gewebeschnitten verwendet werden. Die Vorbehandlung entparaffinierter Gewebe durch Trypsin (4), Proteinase K oder hitzeinduzierte Epitopdemaskierung ist erforderlich. Bei der hitzeinduzierten Epitopdemaskierung werden optimale Resultate mit Dako Target Retrieval Solution, Code-Nr. S1700 oder 10 mmol/L Tris-Puffer, 1 mmol/L EDTA, pH 9.0, erzielt. Weniger optimale Resultate werden mit 10 mmol/L Zitratpuffer, pH 6.0, erzielt. Während der Gewebevorbehandlung oder während des anschließenden immunhistochemischen Färbeverfahrens dürfen die Gewebeschnitte nicht austrocknen.

Gefrierschnitte und Zellpräparate: Aufgrund der Kreuzreaktivität mit einigen Cytokeratin-20-negativen Epithelen (z. B. Nierentubuli) eignet sich der Antikörper nicht zur Markierung von Gefrierschnitten und Zellausstrichen (1). Das Färbeverfahren muss vom Anwender validiert werden.

**Färbeverfahren**

Diese Angaben sind nur Richtlinien. Optimale Bedingungen können je nach Gewebetyp und Vorbereitungsverfahren unterschiedlich sein und sollten vom jeweiligen Labor selbst ermittelt werden. Die Leistung dieses Antikörpers sollte vom Benutzer bei einem Einsatz mit anderen manuellen Färbesystemen oder automatisierten Systemen ermittelt werden.

Verdünnung: Monoclonal Mouse Anti-Human Cytokeratin 20, Code-Nr. M7019, kann auf formalinfixierten, paraffineingebetteten Schnitten von menschlichem Dickdarmgewebe bei einer hitzeinduzierten Epitopdemaskierung von 20 Minuten in Dako Target Retrieval Solution, Code-Nr. S1700, und einer 30-minütigen Inkubation mit dem primären Antikörper bei Raumtemperatur in einem Verdünnungsbereich zwischen 1:25 und 1:50 verwendet werden. Als Negativkontrolle wird Dako Mouse IgG2a, Code-Nr. X0943 empfohlen, das auf dieselbe Konzentration von Maus-IgG wie der primäre Antikörper verdünnt wurde. Falls die Stabilität des verdünnten Antikörpers und der Negativkontrolle für das verwendete Färbeverfahren nicht erwiesen ist, wird empfohlen, diese Reagenzien unmittelbar vor der Verwendung bzw. in Dako Antibody Diluent, Code-Nr. S0809, zu verdünnen.

Qualitätskontrolle: Positiv- und Negativkontrollgewebe sowie Negativ-Kontrollreagenz sollten zur gleichen Zeit und mit demselben Protokoll wie die Patientengewebe getestet werden.

Detektionssystem: Dako EnVision+/HRP Kits (z. B. Code-Nr. K4005) werden empfohlen. Das für das ausgewählte Detektionssystem beschriebene Verfahren befolgen.

**Produktspezifische Beschränkungen**

CK-20 kann gelegentlich in Adenokarzinomen von Brust und Lunge und in Plattenepithelkarzinomen (1, 3) exprimiert werden. Bis zu 5% CK-20-markierte Zellen können in verschiedenen Geweben auftreten (1).

**Auswertung der Färbung**

Mit diesem Antikörper markierte Zellen weisen ein zytoplasmatisches Färbemuster auf.

**Leistungseigenschaften**

Normalgewebe: Der Antikörper markiert gesundes Urothel (1, 4) und das ausgereifte Epithel entlang der Zotten der Duodenalmukosa (1). In einer Reihe von nicht-epithelialen Geweben, wie z. B. glatter Muskulatur, Blutgefäßwänden, Lymphknoten und Tumorstroma wurde keine CK-20-Immunreaktivität nachgewiesen (1).

Anormale Gewebe: In Dickdarm-Adenokarzinomen markierte der Antikörper 26 von 27 Tumoren (96%). 21 (78%) zeigten eine Färbung in mehr als 50% der Zellen (3). Von 51 Patienten mit Urothelpapillom der Blase zeigten 10 eine Markierung von Urothelzellen an der Oberfläche (normale Markierung) durch den Antikörper. Dagegen zeigten 30 Patienten (73%) CK-20-Expression in allen Zellschichten (anormale Markierung) (4). 3 von 4 metastatischen gastrischen Tumoren wurden durch die Antikörper (5) markiert.

Eine konzentrierte Immunfärbung mit dem Antikörper (weniger als 10% markierte Zellen) wurde bei 9 von 65 Lungentumoren, 1 von 20 Brusttumoren und 2 von 11 Tumoren des Endometriums beobachtet. 1 von 19 Nieren-Klarzellkarzinomen wurde in 10-50% der Zellen (3) markiert. Keines der 10 primären Eierstockkarzinome und keines der 5 Mesotheliome wurde durch den Antikörper (5) markiert.

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**Explanation of symbols / Explication des symboles / Erläuterung der Symbole**

| | | |
|---|---|---|
| REF<br>Catalogue number<br>Référence du catalogue<br>Katalognummer | 2 °C – 8 °C<br>Temperature limitation<br>Limites de température<br>Zulässiger Temperaturbereich | <br>Use by<br>Utiliser avant<br>Verwendbar bis |
| IVD<br>In vitro diagnostic medical device<br>Dispositif médical de diagnostic in vitro<br>In-vitro-Diagnostikum | LOT<br>Batch code<br>Réf. du lot<br>Chargenbezeichnung | <br>Manufacturer<br>Fabricant<br>Hersteller |
| <br>Consult instructions for use<br>Consulter les instructions d'utilisation<br>Gebrauchsanweisung beachten | EC REP<br>Authorized representative in the European Community<br>Représentant agréé dans la Communauté européenne<br>Autorisierte Vertretung in der Europäischen Gemeinschaft | |


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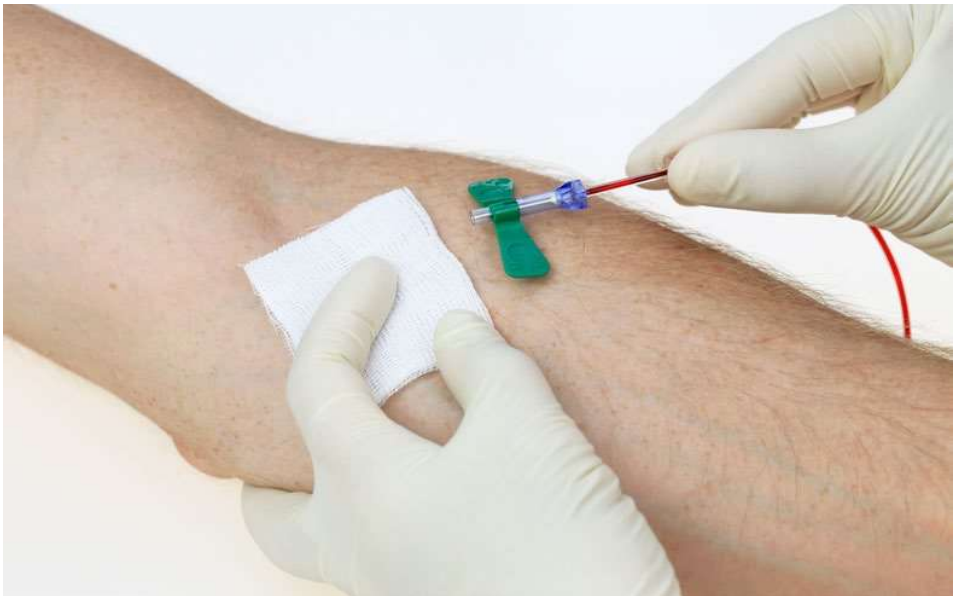

/ You can also find out more about our safety products at www.power4safety.com.

## SAFETY BLOOD COLLECTION SET

**The SAFETY Blood Collection Set was developed especially for patients with difficult vein conditions, but is also suitable for any other blood collection situation, as it offers the patient a particularly gentle collection comfort.**

The product offers a high level of safety by manually activating the protective mechanism while the needle is still inside the vein. The correct activation by the user is indicated by a clearly audible click. The safety blood collection set thus provides the best possible protection against needlestick injuries. The visual control of the puncture through a transparent viewing window also increases the puncture safety. The product can be used for blood collection or for infusions for up to five hours.
The SAFETY Blood Collection Set is a sterile single use winged needle set connected to a flexible tube, with or without a Luer adapter or Luer adapter with holder. The product is ergonomically and individually packed, making it easy to open and causing little waste.

- A viewing window between the needle and the tube shows whether the venipuncture was successful
- Tube with different lengths offers more flexibility in blood collection
- Simple activation of the safety mechanism


## SAFETY Blood Collection/Infusion Set

This version of the product can be used for blood collection by assembling it with, for example, a HOLDEX® or VACUETTE® SAFELINK, or for short-term infusion for up to 5 hours instead.

*Safety mechanism: manual activation*

| Item no. | Colour code | Needle size | Needle length | Needle wall type | Tubing length | Tubing length | Qty. inner / outer |
|---|---|---|---|---|---|---|---|
| 450091 | green | 21 G | 19 mm | thin wall | 7.5″ | 19 cm | 50 / 1,000 |
| 450191 | green | 21 G | 19 mm | thin wall | 12″ | 30 cm | 50 / 1,000 |
| 450092 | blue | 23 G | 19 mm | thin wall | 7.5″ | 19 cm | 50 / 1,000 |
| 450090 | orange | 25 G | 19 mm | thin wall | 7.5″ | 19 cm | 50 / 1,000 |


## SAFETY Blood Collection Set + Luer Adapter

This version of the SBC set has a Luer adapter and can be used with a holder such as our standard tube holder or blood culture holder.

*Safety mechanism: manual activation*

| Item no. | Colour code | Needle size | Needle length | Needle wall type | Tubing length | Tubing length | Qty. inner / outer |
|---|---|---|---|---|---|---|---|
| 450083 | green | 21 G | 19 mm | thin wall | 4″ | 10 cm | 50 / 1,000 |
| 450081 | green | 21 G | 19 mm | thin wall | 7.5″ | 19 cm | 50 / 1,000 |
| 450095 | green | 21 G | 19 mm | thin wall | 12″ | 30 cm | 50 / 1,000 |
| 450084 | blue | 23 G | 19 mm | thin wall | 4″ | 10 cm | 50 / 1,000 |
| 450082 | blue | 23 G | 19 mm | thin wall | 7.5″ | 19 cm | 50 / 1,000 |
| 450096 | blue | 23 G | 19 mm | thin wall | 12″ | 30 cm | 50 / 1,000 |
| 450099 | orange | 25 G | 19 mm | thin wall | 12″ | 30 cm | 50 / 1,000 |


## SAFETY Blood Collection Set + Holder

This version of the SBC set is manufactured with a pre-assembled tube holder and therefore allows an immediate start of the blood collection without assembling with further components.

*Safety mechanism: manual activation*

| Item no. | Colour code | Needle size | Needle length | Needle wall type | Tubing length | Tubing length | Qty. inner / outer |
|---|---|---|---|---|---|---|---|
| 450087 | green | 21 G | 19 mm | thin wall | 4″ | 10 cm | 24 / 240 |
| 450085 | green | 21 G | 19 mm | thin wall | 7.5″ | 19 cm | 24 / 240 |
| 450160 | green | 21 G | 19 mm | thin wall | 12″ | 30 cm | 24 / 240 |
| 450088 | blue | 23 G | 19 mm | thin wall | 4″ | 10 cm | 24 / 240 |
| 450086 | blue | 23 G | 19 mm | thin wall | 7.5″ | 19 cm | 24 / 240 |
| 450161 | blue | 23 G | 19 mm | thin wall | 12″ | 30 cm | 24 / 240 |


## SAFETY Blood Collection Set + Blood Culture Holder

This version of the SBC set is manufactured with a pre-assembled blood culture holder. The product is suitable for blood collection with common blood culture bottles and subsequently with VACUETTE® Blood Collection Tubes if required.

*Safety mechanism: manual activation*

| Item no. | Colour code | Needle size | Needle length | Needle wall type | Tubing length | Tubing length | Qty. inner / outer |
|---|---|---|---|---|---|---|---|
| 450182 | green | 21 G | 19 mm | thin wall | 7.5″ | 19 cm | 24 / 240 |
| 450183 | blue | 23 G | 19 mm | thin wall | 7.5″ | 19 cm | 24 / 240 |
| 450184 | green | 21 G | 19 mm | thin wall | 12″ | 30 cm | 24 / 240 |
| 450185 | blue | 23 G | 19 mm | thin wall | 12″ | 30 cm | 24 / 240 |

# Monoclonal Mouse Anti-Human Melan-A
Clone A103

## Code M7196

## ENGLISH

Technines specifikacijos 36 reikalavimas

**Intended use**
For in vitro diagnostic use.

Monoclonal Mouse Anti-Human Melan-A, Clone A103, is intended for use in immunohistochemistry (IHC). The antibody labels melanocytes and is a useful aid for the classification of melanomas (1, 2), adrenocortical carcinomas (3, 4) and angiomyolipomas (5). Differential classification is aided by the results from a panel of antibodies. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

**Synonyms**
MART-1 (1).

**Summary and explanation**
Melan-A, isolated as a melanoma-specific antigen, is a transmembrane protein composed of 118 amino acids with uncertain function (1). The *Melan-A* gene was cloned from a human melanoma cell line, and its expression on melanomas is recognized by autologous cytotoxic T cells (6). Melan-A is expressed in skin, retina and the majority of cultured melanocytes and melanomas, whereas a vast variety of other tissues and cancers tested do not express Melan-A (1, 6, 7). However, Melan-A may be found expressed in angiomyolipomas (5). Along with Melan-A, seven other melanoma antigens have been identified: MAGE-1, MAGE-3, tyrosinase, gp100, gp75, BAGE-1 and GAGE-1, which are all recognized by autologous cytotoxic T cells (1).

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure; Materials Required, Not Supplied; Storage; Specimen Preparation; Staining Procedure; Quality Control; Troubleshooting; Interpretation of Staining; General Limitations.

**Reagent provided**
Monoclonal mouse antibody provided in liquid form as cell culture supernatant dialysed against 0.05 mol/L Tris-HCl, pH 7.2, and containing 15 mmol/L $NaN_3$.

Clone: A103 (1). Isotype: IgG1, kappa.

Mouse IgG concentration: See label on vial.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

**Immunogen**
Recombinant protein expressed in *E. coli* corresponding to Melan-A (1).

**Specificity**
In Western blotting of cell lysates from Melan-A mRNA positive cell lines, the antibody labels a protein doublet of 20-22 kDa, whereas no labeling is detected in Melan-A mRNA-negative cell lines (1).

In immunoprecipitates of Melan-A-positive cell lines SK-MEL-13 and SK-MEL-19, the antibody labels a protein doublet of 20-22 kDa corresponding to Melan-A, whereas no precipitation is detected in Melan-A mRNA-negative melanoma cell lines (1).

**Precautions**
1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution should be disposed of according to local, State and Federal regulations.

**Storage**
Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

**Specimen preparation**
Paraffin sections: The antibody can be used for labeling paraffin-embedded tissue sections fixed in formalin. Pre-treatment of deparaffinized tissues with heat-induced epitope retrieval is required. Optimal results are obtained with 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0. The following solutions Dako Target Retrieval Solution, Code S1700 and 10 mmol/L citrate buffer, pH 6.0 or pre-treatment of tissues with proteinase K was found inefficient. The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, it is recommended to dilute these reagents immediately before use, or dilute in Dako Antibody Diluent, Code S0809.

Frozen sections and cell preparations: The antibody can be used for labeling acetone-fixed, frozen sections and cell preparations (1). The user must validate the staining procedure.

Technines specifikacijos 36 reikalavimas

**Staining procedure**
These are guidelines only. Optimal conditions may vary depending on specimen type and preparation method, and should be validated individually by each laboratory. The performance of this antibody should be established by the user when utilized with other manual staining systems or automated platforms.

Dilution: Monoclonal Mouse Anti-Human Melan-A, Code M7196, may be used at a dilution range of 1:25-1:50 when applied on formalin-fixed, paraffin-embedded sections of human malignant melanoma and using 20 minutes heat-induced epitope retrieval in 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0, and 30 minutes incubation at room temperature with the primary antibody. The recommended negative control is Dako Mouse IgG1, Code X0931, diluted to the same mouse IgG concentration as the primary antibody.

Visualization: Dako EnVision+ /HRP kits, e.g. Code K4005, are recommended. Follow the procedure enclosed with the selected visualization kit.

Quality control: Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens.

**Product-specific limitations**
Due to nonspecific immunoreactivity, the antibody has been reported to label adrenal cortex, adrenocortical adenomas, primary and metastatic adrenocortical carcinomas (ACC), angiomyolipomas (AML) (5), Leydig cells and Leydig tumors of the testis and granulosa and theca cells of the ovary as well as Sertoli-Leydig cell tumors of the ovary (2, 3).

Using 10 mmol/L Tris buffer, 1 mmol/L EDTA, pH 9.0 for heat-induced epitope retrieval, the antibody may display background staining in single cells of the kidney.

**Staining interpretation**
Cells labeled by the antibody display a cytoplasmic staining pattern (1).

**Performance characteristics**
Normal tissues: The antibody labels skin, whereas stomach, colon, lung, liver, spleen, kidney, testis, urinary bladder, breast, ovary, smooth muscle and adipose tissues are not labeled by the antibody (1, 5). The steroid-producing cells of adrenal cortex, ovary and testis have been reported to be labeled by the antibody (2). See also product-specific limitations.

Abnormal tissues: In metastatic melanomas 16/21 cases were labeled by the antibody, showing homogeneous, cytoplasmic staining in >80-90% of melanoma cells, except one which displayed focal staining (1). In another study of 10 benign melanocytic nevi, 10 primary melanomas and 75 metastatic melanomas, the antibody labeled 10/10 benign melanocytic nevi, 7/10 primary melanomas and 61/75 metastatic melanomas (2).

Among 111 carcinomas, mainly adenocarcinomas and squamous cell carcinomas, 40 germ cell tumors and 33 miscellaneous non-melanocytic epithelioid tumors, none were labeled by the antibody. The antibody labeled 5/5 adrenal cortical adenomas, 16/16 primary and 13/13 metastatic adrenal cortical carcinomas, and also 4/4 Leydig cell tumors of the testis and 3/4 Sertoli-Leydig cell tumors of the ovary were labeled by the antibody (3). In another study of 316 cases, including 21 adrenal cortical tumors, 16 metastatic carcinomas to the adrenal, 10 pheochromocytomas, and 269 extra-adrenal carcinomas, the antibody labeled 14/14 adrenal cortical adenomas, 7/7 adrenal cortical carcinomas, 0/16 metastatic carcinomas to the adrenal and 0/10 pheochromocytomas. Of the 269 extra-adrenal carcinomas, a single ovarian serous carcinoma was labeled (4).

In a study of 18 angiomyolipomas, all 18 were labeled by the antibody (5). In another report all three angiomyolipomas tested were labeled by the antibody (2).

## FRANÇAIS

**Utilisation prévue**
Pour utilisation diagnostique in vitro.

L'anticorps Monoclonal Mouse Anti-Human Melan-A, Clone A103, est destiné à être utilisé en immunohistochimie (IHC). L'anticorps marque les mélanocytes et constitue une aide utile pour la classification des mélanomes (1, 2), des carcinomes corticosurrénaux (3, 4) et des angiomyolipomes (5). La classification différentielle est facilitée par les résultats provenant d'un panel d'anticorps. L'interprétation clinique de toute coloration ou son absence doit être complétée par des études morphologiques en utilisant des contrôles appropriés et doit être évaluée en fonction des antécédents cliniques du patient et d'autres tests diagnostiques par un pathologiste qualifié. Cet anticorps est destiné à être utilisé après un diagnostic primaire de tumeur par histopathologie traditionnelle utilisant des colorations histochimiques non immunologiques.

**Synonymes**
MART-1 (1).

**Résumé et explication**
Le Melan-A, isolé en tant qu'antigène spécifique des mélanomes, est une protéine transmembranaire constituée de 118 acides aminés dont la fonction est incertaine (1). Le gène *Melan-A* a été cloné à partir d'une lignée cellulaire de mélanome humain, et son expression sur les mélanomes est reconnue par les lymphocytes T cytotoxiques autologues (6). Le Melan-A est exprimé dans la peau, la rétine et la majorité des mélanocytes et mélanomes mis en culture, tandis qu'une grande variété d'autres tissus et de cancers testés n'expriment pas le Melan-A (1, 6, 7). Cependant, le Melan-A peut être exprimé dans les angiomyolipomes (5). Outre le Melan-A, sept autres antigènes des mélanomes ont été identifiés : MAGE-1, MAGE-3, tyrosinase, gp100, gp75, BAGE-1 et GAGE-1, qui sont tous reconnus par les lymphocytes T cytotoxiques autologues (1).

Consulter le document *General Instructions for Immunohistochemical Staining* (Instructions générales de coloration immunohistochimique) de Dako ou les instructions du kit de détection pour les procédures IHC : Principe de la procédure, Matériel requis mais non fourni, Conservation, Préparation des échantillons, Procédure de coloration, Contrôle de qualité, Dépannage, Interprétation de la coloration, Limites générales.

**Réactif fourni**
Anticorps monoclonal de souris, fourni sous forme liquide en tant que surnageant de culture cellulaire, dialysé contre 0,05 mol/L de Tris-HCl à pH 7,2 et contenant 15 mmol/L d'azide de sodium ($NaN_3$).

Clone : A103 (1). Isotype : IgG1, kappa.

Concentration en IgG de souris : Voir l'étiquette sur le flacon.

La concentration en protéines peut varier d'un lot à l'autre sans que cela influence la dilution optimale. Le titre de chaque lot est comparé et ajusté par rapport à un lot de référence pour assurer des performances de coloration immunohistochimiques cohérentes d'un lot à l'autre.

**Immunogène**
Protéine recombinante exprimée dans *E. coli* correspondant au Melan-A (1).

**Spécificité**
Lors d'analyse par Western blot de lysats cellulaires provenant de lignées cellulaires positives à l'ARNm du Melan-A, l'anticorps marque un doublet protéique de 20-22 kDa, alors qu'aucun marquage n'est détecté dans les lignées cellulaires négatives à l'ARNm du Melan-A (1).

Dans les immunoprécipités de lignées cellulaires SK-MEL-13 et SK-MEL-19 positives au Melan-A, l'anticorps marque un doublet protéique de 20-22 kDa correspondant au Melan-A, alors qu'aucune précipitation n'est détectée dans les lignées cellulaires négatives à l'ARNm du Melan-A (1).

**Précautions d'emploi**
1. Pour utilisation diagnostique in vitro.
2. Pour utilisateurs professionnels.
3. Ce produit contient de l'azide de sodium ($NaN_3$), un produit chimique hautement toxique à l'état pur. Aux concentrations du produit, bien que non classé comme dangereux, l'azide de sodium peut réagir avec le cuivre et le plomb des canalisations et former des accumulations d'azides métalliques hautement explosives. Lors de l'élimination, rincer abondamment à l'eau pour éviter toute accumulation d'azide métallique dans les canalisations.
4. Comme avec tout produit d'origine biologique, des procédures de manipulation appropriées doivent être respectées.
5. Porter un équipement de protection individuelle approprié pour éviter tout contact avec les yeux et la peau.
6. Les solutions non utilisées doivent être éliminées conformément aux réglementations locales, nationales et européennes.

**Conservation**
Conserver entre 2 et 8 °C. Ne pas utiliser après la date de péremption imprimée sur le flacon. Si les réactifs sont conservés dans des conditions autres que celles indiquées, celles-ci doivent être validées par l'utilisateur. Il n'existe pas de signe particulier pour indiquer l'instabilité de ce produit. Par conséquent, des contrôles positifs et négatifs doivent être testés en même temps que les échantillons de patient. Si une coloration inattendue est observée, qui ne peut être expliquée par des différences dans les procédures du laboratoire et qu'un problème lié à l'anticorps est suspecté, contacter l'assistance technique de Dako.

**Préparation des échantillons**
Coupes en paraffine : L'anticorps peut être utilisé pour le marquage des coupes de tissus incluses en paraffine et fixées au formol. Le prétraitement des tissus déparaffinés avec une restauration d'épitope induite par la chaleur est nécessaire. Des résultats optimaux sont obtenus dans un tampon Tris à 10 mmol/L, EDTA à 1 mmol/L, à pH 9,0. Toutefois, la Dako Target Retrieval Solution, réf. S1700 et le tampon citrate à 10 mmol/L à pH 6,0 ou le prétraitement des tissus par la protéinase K se sont avérés inefficaces. Les coupes de tissus ne doivent pas sécher lors du traitement ni lors de la procédure de coloration immunohistochimique suivante. À moins que la stabilité de l'anticorps dilué et du contrôle négatif n'ait été établie dans la procédure de coloration en cours, il est recommandé de diluer ces réactifs immédiatement avant utilisation ou de les diluer avec le produit Dako Antibody Diluent, réf. S0809.

Coupes congelées et préparations cellulaires : L'anticorps peut être utilisé pour le marquage des coupes congelées et fixées à l'acétone et des préparations cellulaires (1). L'utilisateur doit valider la procédure de coloration.

**Procédure de coloration**
Il ne s'agit là que de conseils. Les conditions optimales peuvent varier en fonction du type de prélèvement et de la méthode de préparation, et doivent être validées individuellement par chaque laboratoire. Les performances de cet anticorps doivent être établies par l'utilisateur lorsqu'il est utilisé avec d'autres systèmes de coloration manuelle ou plates-formes automatisées.

Dilution : Le Monoclonal Mouse Anti-Human Melan-A, réf. M7196, peut être utilisé à une gamme de dilution de 1:25-1:50 lorsqu'il est appliqué sur des coupes de mélanome malin humain fixées au formol et incluses en paraffine, en utilisant une restauration d'épitope induite par la chaleur de 20 minutes dans un tampon Tris à 10 mmol/L, EDTA à 1 mmol/L, à pH 9,0 et une incubation de 30 minutes avec l'anticorps primaire à température ambiante. Le contrôle négatif recommandé est le produit Dako Mouse IgG1, réf. X0931, dilué à la même concentration en IgG de souris que l'anticorps primaire.

Visualisation : Il est recommandé d'utiliser les kits Dako EnVision+/HRP, par exemple la réf. K4005. Suivre la procédure incluse dans le kit de visualisation sélectionnée.

Contrôle de qualité : Les tissus de contrôle positifs et négatifs, ainsi que le réactif de contrôle négatif, doivent être testés en parallèle selon le même protocole que pour les échantillons de patients.

**Limitations spécifiques du produit**
En raison de l'immunoréactivité non spécifique, il a été rapporté que l'anticorps marque le cortex surrénal, les adénomes corticosurrénaux, et les carcinomes métastatiques corticosurrénaux (ACC), les angiomyolipomes (LMA) (5), les cellules de Leydig et les tumeurs de Leydig du testicule et de la granulosa et les cellules thécales de l'ovaire ainsi que les tumeurs des cellules de Sertoli-Leydig de l'ovaire (2, 3).

A l'aide du tampon Tris à 10 mmol/L, EDTA à 1 mmol/L, pH 9,0 pour la restauration d'épitope induite par la chaleur, l'anticorps peut présenter une coloration de fond dans les cellules individuelles du rein.

**Interprétation de la coloration**
Les cellules marquées par l'anticorps présentent un motif de coloration cytoplasmique (1).

**Performances**
Tissus sains : L'anticorps marque la peau, alors que l'estomac, le côlon, le poumon, le foie, la rate, le rein, le testicule, la vessie, le sein, l'ovaire, les cellules de muscle lisse et les tissus adipeux ne sont pas marqués par l'anticorps (1, 5). Il a été signalé que les cellules productrices de stéroïdes du cortex surrénal, de l'ovaire et du testicule étaient marquées par l'anticorps (2). Voir également les limites spécifiques du produit.

Tissus anormaux : Pour les mélanomes métastatiques, 16 cas sur 21 ont été marqués par l'anticorps, avec un marquage cytoplasmique homogène dans > 80 à 90% des cellules de mélanomes, à l'exception d'un cas qui a présenté une coloration focale (1). Lors d'une autre étude de 10 nævi mélanocytaires bénins, 10 mélanomes primaires et 75 mélanomes métastatiques, l'anticorps a marqué 10 nævi mélanocytaires bénins sur 10, 7 mélanomes primaires sur 10 et 61 mélanomes métastatiques sur 75 (2).

Sur 111 carcinomes, principalement des adénocarcinomes et des carcinomes à cellules squameuses, 40 tumeurs à cellules germinales et 33 tumeurs épithélioïdes non mélanocytaires diverses, aucun marquage par l'anticorps n'a été observé. L'anticorps a marqué 5 adénomes corticosurrénaux sur 5, 16 carcinomes corticosurrénaux primaires sur 16 et 13 carcinomes corticosurrénaux métastatiques sur 13, ainsi que 4 tumeurs des cellules de Leydig du testicule sur 4 et 3 tumeurs des cellules de Sertoli- Leydig de l'ovaire sur 4 (3). Lors d'une autre étude portant sur 316 cas, dont 21 tumeurs corticosurrénales, 16 carcinomes métastatiques de la surrénale, 10 phéochromocytomes, et 269 carcinomes extra-surrénaux, l'anticorps a marqué 14 adénomes corticosurrénaux sur 14, 7 carcinomes corticosurrénaux sur 7, 0 carcinome métastatique de la surrénale sur 16 et 0 phéochromocytome sur 10. Parmi les 269 carcinomes extra-surrénaux, un seul carcinome séreux de lovaire a été marqué (4).

Lors d'une étude, 18 angiomyolipomes sur 18 ont été marqués par l'anticorps (5). Dans un autre rapport, les trois angiomyolipomes testés ont été marqués par l'anticorps (2).

## DEUTSCH

**Verwendungszweck**
Zur In-vitro-Diagnostik.

Monoclonal Mouse Anti-Human Melan-A, Clone A103, ist zur Verwendung in der Immunhistochemie (IHC) bestimmt. Der Antikörper markiert Melanozyten und unterstützt die Klassifizierung von Melanomen (1, 2), adrenokortikalen Karzinomen (3, 4) und Angiomyolipomen (5). Die Differenzialklassifikation wird durch die Ergebnisse eines Antikörper-Panels unterstützt. Die klinische Auswertung einer eintretenden oder ausbleibenden Färbung sollte durch morphologische Studien mit geeigneten Kontrollen ergänzt werden und von einem qualifizierten Pathologen unter Berücksichtigung der Krankengeschichte und anderer diagnostischer Tests des Patienten vorgenommen werden. Dieser Antikörper kommt nach der Primärdiagnose des Tumors durch konventionelle Histopathologie unter Verwendung von nicht immunologischen histochemischen Färbungen zum Einsatz.

**Synonyme**
MART-1 (1).

**Zusammenfassung und Erklärung**
Melan-A, isoliert als ein melanomspezifisches Antigen, ist ein Transmembranprotein mit ungeklärter Funktion, das aus 118 Aminosäuren besteht (1). Das *Melan-A*-Gen wurde aus einer menschlichen Melanomzelllinie geklont, und seine Expression auf Melanomen wird von autologen zytotoxischen T-Zellen erkannt (6). Melan-A wird von Zellen der Haut, der Netzhaut und dem Großteil der Melanozyten und Melanome Zellkultur exprimiert, während der weit überwiegende Teil anderer untersuchter Gewebe und Karzinome Melan-A nicht exprimiert (1, 6, 7). Melan-A kann jedoch in Angiomyolipomen exprimiert werden (5). Neben Melan-A wurden noch sieben weitere Melanomantigene identifiziert: MAGE-1, MAGE-3, Tyrosinase, gp100, gp75, BAGE-1 und GAGE-1. Diese werden alle von autologen zytotoxischen T-Zellen erkannt (1).

Folgende Angaben bitte den *General Instructions for Immunohistochemical Staining* (Allgemeine Richtlinien zur immunhistochemischen Färbung) von Dako bzw. den Anweisungen des Detektionssystems für IHC-Verfahren entnehmen: Verfahrensprinzipien, Erforderliche, aber nicht mitgelieferte Materialien, Lagerung, Gewebevorbereitung, Färbeverfahren, Qualitätskontrolle, Fehlerbehandlung, Auswertung der Färbung, Allgemeine Beschränkungen.

**Geliefertes Reagenz**
Monoklonale Mausantikörper in flüssiger Form als gegen 0.05 mol/L Tris-HCl, pH 7.2 und 15 mmol/L $NaN_3$ dialysierter Zellkulturüberstand.

Klon: A103 (1). Isotyp: IgG1, Kappa.

Konzentration von Maus-IgG: Siehe Behälteretikett.

Die Proteinkonzentration kann zwischen Chargen abweichen, ohne die optimale Verdünnung zu beeinflussen. Der Titer jeder Charge wird mit dem einer Referenzcharge verglichen und dieser angeglichen, um konstante immunhistochemische Färbeergebnisse zwischen den Chargen zu gewährleisten.

**Immunogen**
Rekombinantes Protein, in *E. coli* exprimiert und Melan-A entsprechend (1).

**Spezifität**
Beim Western-Blotting von Zelllysaten aus Melan-A-mRNA-positiven Zelllinien markiert der Antikörper ein Proteinpaar von 20-22 kDa, während in Melan-A-mRNA-negativen Zellinien keine Markierung nachgewiesen werden konnte (1).

In Immunpräzipitaten von Melan-A-positiven Zelllinien SK-MEL-13 und SK-MEL-19 markiert der Antikörper ein Melan-A entsprechendes Proteinpaar mit einer molekularen Masse von 20-22 kDa, während in Melanom-Zelllinien, welche Melan-A-mRNA-negativ sind, keine Präzipitation nachgewiesen wird (1).

**Vorsichtsmaßnahmen**
1. Zur In-vitro-Diagnostik.
2. Für Fachpersonal.
3. Dieses Produkt enthält Natriumazid ($NaN_3$), eine in reiner Form äußerst giftige Chemikalie. Bei den in diesem Produkt verwendeten Konzentrationen kann Natriumazid, obwohl nicht als gefährlich klassifiziert, mit in Wasserleitungen vorhandenem Blei oder Kupfer reagieren und zur Bildung von hochexplosiven Metallazid-Anreicherungen führen. Nach der Entsorgung muss mit reichlich Wasser nachgespült werden, um Metall-Azid-Anreicherung zu vermeiden.
4. Wie alle Produkte biologischen Ursprungs müssen auch diese entsprechend gehandhabt werden.
5. Geeignete persönliche Schutzausrüstung (PSA) tragen, um Augen- und Hautkontakt zu vermeiden.
6. Nicht verwendete Lösung ist entsprechend örtlichen, staatlichen und EU-rechtlichen Richtlinien zu entsorgen.

**Lagerung**
Bei 2-8 °C lagern. Nach Ablauf des auf dem Behälter aufgedruckten Verfallsdatums nicht mehr verwenden. Werden die Reagenzien unter anderen als den angegebenen Bedingungen aufbewahrt, müssen diese Bedingungen vom Benutzer überprüft werden. Es gibt keine offensichtlichen Anhaltspunkte für die mögliche Instabilität dieses Produkts. Es sollten daher die Positiv- und Negativkontrollen gleichzeitig mit den Patientengewebeproben mitgeführt werden. Wenn eine unerwartete Anfärbung beobachtet wird, welche durch Änderungen in den Labormethoden nicht erklärt werden kann, und falls Verdacht auf ein Problem mit dem Antikörper besteht, ist Kontakt mit dem technischen Kundendienst von Dako aufzunehmen.

**Gewebevorbereitung**
Paraffinschnitte: Der Antikörper kann für die Markierung von formalinfixierten, paraffineingebetteten Gewebeschnitten verwendet werden. Die Vorbehandlung des Gewebes durch hitzeinduzierte Epitopdemaskierung ist erforderlich. Optimale Resultate werden mit 10 mmol/L Tris-Puffer, 1 mmol/L EDTA, pH 9.0, erzielt. Dako Target Retrieval Solution, Code-Nr. S1700, und 10 mmol/L Citratpuffer, pH 6.0, sowie die Vorbehandlung von Gewebe mit Proteinase K erwiesen sich als ineffizient. Während der Gewebevorbehandlung oder während des anschließenden immunhistochemischen Färbeverfahrens dürfen die Gewebeschnitte nicht austrocknen. Falls die Stabilität des

verdünnten Antikörpers und der Negativkontrolle für das verwendete Färbeverfahren nicht erwiesen ist, wird empfohlen, diese Reagenzien unmittelbar vor der Verwendung bzw. in Dako Antibody Diluent, Code-Nr. S0809, zu verdünnen.

Gefrierschnitte und Zellpräparate: Der Antikörper eignet sich zur Markierung von azetonfixierten Gefrierschnitten und Zellpräparaten (1). Das Färbeverfahren muss vom Anwender validiert werden.

**Färbeverfahren**
Diese Angaben sind nur Richtlinien. Optimale Bedingungen können je nach Gewebetyp und Vorbereitungsverfahren unterschiedlich sein und sollten vom jeweiligen Labor selbst ermittelt werden. Die Leistung dieses Antikörpers sollte vom Benutzer bei einem Einsatz mit anderen manuellen Färbesystemen oder automatisierten Systemen ermittelt werden.

Verdünnung: Monoclonal Mouse Anti-Human Melan-A, Code-Nr. M7196, kann auf formalinfixierten, paraffineingebetteten Schnitten von humanem malignem Melanom bei einer hitzeinduzierten Epitopdemaskierung von 20 Minuten in 10 mmol/L Tris-Puffer, 1 mmol/L EDTA, pH 9.0, und einer 30-minütigen Inkubation mit dem primären Antikörper bei Raumtemperatur in einem Verdünnungsbereich zwischen 1:25 und 1:50 verwendet werden. Als Negativkontrolle wird Dako Mouse IgG1, Code-Nr. X0931, empfohlen, das auf dieselbe Konzentration an Maus-IgG wie der primäre Antikörper verdünnt wurde.

Detektionssystem: Dako EnVision+/HRP Kits (z. B. Code-Nr. K4005) werden empfohlen. Das für das ausgewählte Detektionssystem beschriebene Verfahren befolgen.

Qualitätskontrolle: Positiv- und Negativkontrollgewebe sowie Negativ-Kontrollreagenz sollten zur gleichen Zeit und mit demselben Protokoll wie die Patientengewebe getestet werden.

**Produktspezifische Beschränkungen**
Berichten zufolge markierte der Antikörper aufgrund der unspezifischen Immunreaktivität die Nebennierenrinde, Adenome der Nebennierenrinde, primäre und metastatische Nebennierenrindenkarzinome (ACC), Angiomyolipome (AML) (5), Leydig-Zellen und Leydig-Tumore der Hoden und Granulosa- und Thekazellen der Eierstöcke sowie Sertoli-Leydig-Zelltumore der Eierstöcke (2, 3).

Bei hitzeinduzierter Epitopdemaskierung mit 10 mmol/L Tris-Puffer, 1 mmol/L EDTA, pH 9.0, kann der Antikörper eine Hintergrundfärbung in einzelnen Nierenzellen aufweisen.

**Auswertung der Färbung**
Mit diesem Antikörper markierte Zellen weisen ein zytoplasmatisches Färbemuster auf (1).

**Leistungseigenschaften**
Normalgewebe: Der Antikörper markiert Hautproben; Proben von Magen, Dickdarm, Lunge, Leber, Milz, Niere, Hoden, Harnblase, Brust, Eierstöcken, glatter Muskulatur und Fettgeweben werden dagegen nicht markiert (1, 5). Die steroidproduzierenden Zellen von Nebennierenrinde, Eierstöcken und Hoden wurden Berichten zufolge durch den Antikörper markiert (2). Siehe auch „Produktspezifische Beschränkungen“.

Anormale Gewebe: Bei metastatischen Melanomen wurden 16/21 Fällen durch den Antikörper markiert und zeigten eine homogene zytoplasmatische Färbung in über 80-90% der Melanomzellen. Nur in einem Fall kam es zu einer örtlich begrenzten Färbung (1). In einer weiteren Studie mit 10 benignen Melanozyten-Naevi, 10 primären und 75 metastatischen Melanomen markierte der Antikörper 10/10 Fällen der benignen Melanozyten-Naevi, 7/10 der primären und 61/75 der metastatischen Melanome (2).

Von 111 Karzinomen, hauptsächlich Adenokarzinomen und Plattenepithelkarzinomen, 40 Keimzelltumoren und 33 diversen epitheloiden Nicht-Melanozyten-Tumoren wurde kein einziger durch den Antikörper markiert. Der Antikörper markierte 5/5 Adenomen der Nebennierenrinde, 16/16 primären und 13/13 metastatischen Nebennierenrindenkarzinomen sowie 4/4 Leydigzell-Tumoren des Hodens und 3/4 Sertoli-Leydig-Zell-Tumoren der Eierstöcke (3). In einer weiteren Studie mit 316 untersuchten Fällen, darunter 21 Nebennierenrindentumore, 16 in die Nebenniere metastasierende Karzinome, 10 Phäochromozytome und 269 extraadrenale Karzinome, markierte der Antikörper 14/14 Adenomen der Nebennierenrinde, 7/7 Karzinomen der Nebennierenrinde, 0/16 metastatischen Karzinomen in der Nebenniere und 0/10 Phäochromozytomen. Von den übrigen 269 extraadrenalen Karzinomen wurde nur ein einziger Fall eines serösen Eierstock-Karzinoms markiert (4).

In einer Studie mit 18 Angiomyolipomen wurden alle 18 Fälle durch den Antikörper markiert (5). Ein weiterer Bericht schildert 3 untersuchte Angiomyolipome, welche ausnahmslos durch den Antikörper markiert wurden (2).

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**Explanation of symbols / Explication des symboles / Erläuterung der Symbole**

| | | |
|---|---|---|
| REF<br>Catalogue number<br>Référence du catalogue<br>Katalognummer | 2 °C – 8 °C<br>Temperature limitation<br>Limites de température<br>Zulässiger Temperaturbereich | Use by<br>Utiliser avant<br>Verwendbar bis |
| IVD<br>In vitro diagnostic medical device<br>Dispositif médical de diagnostic in vitro<br>In-vitro-Diagnostikum | LOT<br>Batch code<br>Réf. du lot<br>Chargenbezeichnung | Manufacturer<br>Fabricant<br>Hersteller |
| Consult instructions for use<br>Consulter les instructions d'utilisation<br>Gebrauchsanweisung beachten | EC REP<br>Authorized representative in the European Community<br>Représentant agréé dans la Communauté européenne<br>Autorisierte Vertretung in der Europäischen Gemeinschaft | |


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# Monoclonal Mouse Anti-Human p63 Protein

Clone DAK-p63

**Code M7317**

## ENGLISH

**Intended use**

For in vitro diagnostic use.

Monoclonal Mouse Anti-Human p63 Protein, Clone DAK-p63, is intended for use in immunohistochemistry. Antibodies to p63 protein, a basal epithelial cell proliferation regulator (1), may be useful for the identification of prostate adenocarcinoma as an aid in the differentiation between benign prostate lesions and prostate adenocarcinoma (2, 3). Antibodies to p63 may also be useful as an aid in the differentiation between breast carcinoma in situ and breast carcinoma (4), to differentiate squamous cell carcinoma from adenocarcinoma of the lung (5, 6) and to differentiate uterine cervical squamous carcinoma from cervical adenocarcinoma (7). The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

**Synonyms for antigen**

Tumor protein (p63).

**Summary and explanation**

The p63 protein is a member of the p53 family, which also includes p73. The p63 gene encodes multiple isoforms: isoforms containing a potent amino-terminal transactivation domain (TAp63 isoforms) and isoforms lacking that region (ΔNp63 isoforms) (8, 9). Although the TAp63 isoforms can transactivate p53 target genes, e.g. Bax and $p21^{WAF/CEP1}$ and induce apoptosis and cell cycle arrest (10), p63 is not a tumor suppressor (9). The ΔNp63 isoforms act in a dominant-negative manner by competing for the p53 target genes and indirectly promote cell growth by counteracting the apoptosis and cell cycle arrest activation by TAp63 isoforms and p53 (1, 10, 11).

P63 is a marker of non-invasive epithelial tumors, whereas loss of p63 expression is seen in more invasive tumors suggesting that loss of p63 accelerates tumorigenesis and metastasis (10). However, a lack of p63 is not a reliable marker of invasiveness and even though p63 is expressed in the minority of breast carcinomas, rare cases of nuclear p63 expression are found (9).

Frequently, tumors have simultaneous transcriptional up-regulation of both the TAp63 and the ΔNp63 isoforms, with ΔNp63 being the predominant at protein level. Some lung cancers and squamous cell carcinomas of the head and neck show p63 protein overexpression associated with a modest increase in p63 gene copy numbers, but the major p63 isoforms are ΔNp63 isoforms. Similarly, in nasopharyngeal carcinomas and esophageal squamous cell carcinoma, ΔNp63 isoforms are the major isotypes (9).

The predominant localization of p63 protein is in basal cells of normal epithelia in ectocervix, esophagus, prostate, skin, tonsil, urothelium, and vagina and in basal cells in glandular structures of breast, bronchi and prostate. p63 protein is also expressed in myoepithelial cells of the breast (9).

Refer to Dako's *General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures.

**Reagent provided**

Monoclonal mouse antibody provided in liquid form as cell culture supernatant (containing fetal calf serum) dialyzed against 0.05 mol/L Tris/HCl, pH 7.2, and containing 0.015 mol/L sodium azide

Clone: DAK-p63. Isotype: IgG2a, kappa.

Mouse IgG concentration mg/L: See label on vial.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

**Immunogen**

Synthetic peptide derived from the core DNA-binding domain of human p63 protein.

**Specificity**

In Western blot analysis the antibody detects bands corresponding to the expected molecular weights and according to expression patterns of the various isoforms (TAp63 and ΔNp63 isoforms) of p63 in HCC1806 squamous carcinoma lysate and colon cancer.

**Precautions**

1. For professional users.
2. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused solution should be disposed of according to local, State and Federal regulations.

**Storage**

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

**Quick guide***

| Step | | Comments |
|---|---|---|
| Fixation | Formalin | |
| Pre-treatment | EnVision FLEX™, High pH (Code K8004) | 20 min HIER, 3-in-1 using PT Link and PT Link Rinse Station |
| Dilution | 1:50 | 20 min incubation |
| Dilution Buffer | Dako Antibody Diluent (Code S0809) | Dilute immediately prior to use |
| Negative Control | Dako Negative Control, Mouse IgG2a (Code X0943) | 20 min incubation |
| Visualization | EnVision™ FLEX, High pH (Code K8000/K8010) | 20 min incubation, 2x5 min DAB+ incubation |
| Counterstain | EnVision™ FLEX Hematoxylin (Code K8008/K8018) | 5 min incubation |
| Control Tissue | Tonsil, prostate | Nuclear staining |
| Slides | FLEX IHC Microscope Slides (Code K8020) | Recommended for greater adherence of tissue sections to glass slides. |

| | | |
|---|---|---|
| Mounting | Non-aqueous, permanent mounting required | After staining, the sections must be dehydrated, cleared and mounted using permanent mounting medium. |
| Instrumentation | Autostainer Link 48 and Autostainer Plus | Use instrument-specific vials (Code SK200-SK203 and Code S3425) |

**The user must always read the package insert for detailed instructions of the staining procedure and handling of the product.*

**Specimen preparation**

Paraffin sections: The antibody can be used for labeling paraffin-embedded tissue sections fixed in formalin. Tissue specimens should be cut into sections of approximately 4 µm.

Pre-treatment: Pre-treatment of formalin-fixed, paraffin-embedded tissue sections with heat-induced epitope retrieval (HIER) is required. Optimal results are obtained by pretreating tissues with HIER using diluted EnVision™ FLEX Target Retrieval Solution, High pH (50x) (Code K8004). Deparaffinization, rehydration and epitope retrieval can be performed in Dako PT Link (Code PT100/PT101). For details, please refer to PT Link User Guide. The following parameters should be used for PT Link: Pre-heat temperature: 65 °C; epitope retrieval temperature and time: 97 °C for 20 (±1) minutes; cool down to 65 °C. Remove slide rack from PT tank and immediately dip slides in jar/tank (e.g., PT Link Rinse Station (Code PT109)) containing diluted room temperature EnVision™ FLEX Wash Buffer (20x) (Code K8007). Leave slides in Wash Buffer for 1-5 minutes.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides (Code K8020) is recommended. After staining, the sections must be dehydrated, cleared and mounted using permanent mounting medium.

**Staining procedure**

Dilution: The recommended dilution of Monoclonal Mouse Anti-Human p63 Protein, Clone DAK-p63, Code M7317, is 1:50. Dilute the antibody in Dako Antibody Diluent (Code S0809). Incubate pretreated tissue sections for 20 minutes at room temperature. These are guidelines only. Optimal conditions may vary depending on specimen and preparation method, and should be validated individually by each laboratory.

Negative control: The recommended negative control reagent Dako Negative Control, Mouse IgG2a (Code X0943), diluted to the same Ig concentration as the primary antibody. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, dilute these reagents immediately prior to use. Positive and negative controls should be run simultaneously with patient specimens.

Visualization: The recommended visualization system is EnVision™ FLEX, High pH (Code K8000/K8010) using a 20 minute incubation at room temperature. Follow the procedure enclosed with the selected visualization system(s).

Automation: The antibody is well-suited for immunohistochemical staining using automated platforms, such as Dako Autostainer, Autostainer Plus and Autostainer Link as well as PT Link for pre-treatment.

Counterstaining: The recommended counterstain is EnVision™ FLEX Hematoxylin (Code K8008/K8018). For optimal results, non-aqueous, permanent mounting medium is recommended.

Controls: Positive and negative control tissues should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include tonsil and prostate and the cells/structures should display reaction patterns as described for this tissue in the "Performance characteristics" section.

**Staining interpretation**

The cellular staining pattern is nuclear. Cytoplasmic staining in abnormal tissue has also been reported (12).

**Performance characteristics**

Normal tissues: In tonsil, squamous epithelial cells show a moderate to strong staining reaction. In prostate, basal epithelial cells show a weak to moderate staining reaction. Occasionally, cytoplasmic labeling of granulocytes may be observed.

**Summary of Normal Tissue Reactivity (13).**

| Tissue Type (# tested) | Positive Tissue Elements | Tissue Type (# tested) | Positive Tissue Elements |
|---|---|---|---|
| Adrenal (3) | 3/3 Adrenal cells (30%), cytoplasmic | Ovary (3) | 0/3 |
| Bone marrow (3) | 0/3 | Pancreas (3) | 3/3 Islet cells (100%), cytoplasmic |
| Breast (3) | 3/3 Basal cells (90%), nuclear | Pituitary (3) | 3/3 Pituitary cells (100%), cytoplasmic |
| Cerebellum (3) | 0/3 | Placenta (3) | 1/3 Synsytiothrophoblastic cells (10%), nuclear |
| Cerebrum (3) | 0/3 | Prostate (3) | 3/3 Basal cells (100%), nuclear |
| Cervix (3) | 3/3 Basal cells (100%), nuclear | Salivary gland (3) | 3/3 Myoepithelial basal cells (90%), nuclear |
| Colon (3) | 0/3 | Skin (3) | 3/3 Epithelial cells and basal cells (100%), nuclear |
| Endometrium (3) | 0/3 | Small intestine (3) | 2/3 Epithelial cells (10%), cytoplasmic |
| Esophagus (3) | 3/3 Epithelial cells (100%), nuclear | Spinal cord (3) | 0/3 |
| Fallopian tube (3) | 3/3 Basal cells (50%), nuclear | Spleen (3) | 0/3 |
| Kidney (3) | 0/3 | Stomach (3) | 3/3 Glandular cells (30-100%), cytoplasmic |
| Liver (3) | 0/3 | Testis (3) | 0/3 |
| Lung (3) | 1/3 basal cells (100%), nuclear | Thyroid (3) | 0/3 |
| Lymph node (3) | 0/3 | Tonsil (3) | 3/3 Epithelial cells (100%), nuclear |
| Muscle, cardiac (3) | 0/3 | Uterus (3) | 2/3 Epithelial cells (<1%), nuclear |
| Muscle, skeletal (3) | 0/3 | Uretrer (3) | 3/3 Epithelial cells (100%), nuclear |
| Nerve, peripheral (3) | 0/3 | Urinary bladder (3) | 3/3 Epithelial cells (100%), nuclear |

Abnormal tissues: The antibody labeled basal cells in 10/10 prostate hyperplasia and myoepithelial cells in 5/5 breast carcinoma in situ. The antibody labeled 6/6 squamous cell carcinoma of the lung, 6/6 uterine cervical squamous cell carcinoma, 0/10 prostate carcinoma, 3/3 breast carcinoma, 4/6 cervical adenocarcinoma, 4/6 adenocarcinoma of the lung (14).

## FRANÇAIS

**Intérêt**

Pour utilisation diagnostique in vitro.

L'anticorps Monoclonal Mouse Anti-Human p63 Protein, Clone DAK-p63, est destiné à une utilisation en immunohistochimie. Les anticorps dirigés contre la protéine p63, un régulateur de la prolifération des cellules épithéliales basales (1), peuvent s'avérer utiles pour identifier l'adénocarcinome de la prostate et différencier les lésions prostatiques bénignes des adénocarcinomes de la prostate (2, 3). Ils peuvent également être utiles pour différencier le carcinome du sein *in situ* du carcinome du sein (4), le carcinome épidermoïde de l'adénocarcinome pulmonaire (5, 6) et le carcinome épidermoïde du col utérin de l'adénocarcinome cervical (7). L'interprétation clinique de toute coloration ou son absence doit être complétée par des études morphologiques en utilisant des contrôles appropriés et doit être évaluée en fonction des antécédents cliniques du patient et d'autres tests diagnostiques réalisés par un pathologiste.

**Synonymes de l'antigène**

Protéine tumorale (p63).

**Résumé et explication**

La protéine p63 appartient à la famille p53, qui inclut également la p73. Le gène p63 code pour plusieurs isoformes : les isoformes possédant un domaine de transactivation N-terminal puissant (isoformes TAp63) et les isoformes ne possédant pas ce domaine (isoformes ΔNp63) (8, 9). Bien que les isoformes TAp63 puissent transactiver les gènes cibles p53 (par ex., Bax et $p21^{WAF/CEP1}$) et induire une apoptose et un arrêt du cycle cellulaire (10), p63 n'est pas un suppresseur tumoral (9). Les isoformes ΔNp63 ont un effet dominant négatif dans la compétition pour les gènes cibles p53, et ils favorisent indirectement la croissance cellulaire en s'opposant à l'apoptose et l'arrêt du cycle cellulaire par les isoformes TAp63 et p53 (1, 10, 11).

La protéine p63 est un marqueur des tumeurs épithéliales non invasives. L'absence d'expression de p63 s'observe dans les tumeurs davantage invasives, suggérant que la perte de p63 accélère l'oncogénèse et la métastase (10). Néanmoins, l'absence de p63 ne constitue pas une indication fiable d'invasion tumorale et, bien qu'elle ne soit exprimée que dans une minorité de carcinomes du sein, de rares cas d'expression nucléaire de p63 ont été observés (9).

Les tumeurs présentent souvent une régulation positive de la transcription des deux isoformes en même temps (TAp63 et ΔNp63), les isoformes ΔNp63 étant prédominantes au niveau des protéines. Dans certains cancers du poumon et carcinomes épidermoïdes de la tête et du cou, une surexpression de la protéine p63 associée à une augmentation modérée du nombre de copies du gène p63 a été observée, mais les isoformes majoritaires sont les isoformes ΔNp63. De la même manière, les isoformes ΔNp63 sont majoritaires dans les carcinomes nasopharyngés et les carcinomes épidermoïdes de l'œsophage (9).

La protéine p63 est principalement présente dans les cellules basales des épithéliums normaux de l'exocol, de l'œsophage, de la prostate, de la peau, de l'amygdale, de l'urothélium et du vagin, ainsi que dans les cellules basales des structures glandulaires du sein, des bronches et de la prostate. La protéine p63 est également exprimée dans les cellules myoépithéliales du sein (9).

Se référer au document *General Instructions for Immunohistochemical Staining* (Instructions générales de coloration immunohistochimique) de Dako ou aux instructions du système de détection relatives aux procédures IHC.

**Réactif fourni**

Anticorps monoclonal de souris fourni sous forme liquide comme surnageant de culture cellulaire (contenant du sérum fœtal de veau) dialysé en utilisant 0,05 mol/L de tampon Tris-HCl, à pH 7,2 et contenant 0,015 mol/L d'azide de sodium.

Clone : DAK-p63. Isotype : IgG2a, kappa.

Concentration (mg/L) en IgG de souris : Voir l'étiquette sur le flacon.

La concentration de protéines peut varier d'un lot à l'autre sans que cela n'ait d'influence sur la dilution optimale. Le titre de chaque lot est comparé et ajusté par rapport à un lot de référence pour assurer des performances de coloration immunohistochimiques analogues d'un lot à l'autre.

**Immunogène**

Peptide synthétique dérivé du domaine du noyau liant l'ADN de la protéine humaine p63.

**Spécificité**

L'analyse par Western blot d'un lysat de carcinome épidermoïde HCC1806 et de cancer du côlon révèle que l'anticorps détecte des bandes correspondant aux poids moléculaires attendus et aux schémas d'expression des différents isoformes (TAp63 et ΔNp63) de p63.

**Précautions d'emploi**

1. Pour utilisateurs professionnels.
2. Ce produit contient de l'azide de sodium ($NaN_3$), un produit chimique hautement toxique à l'état pur. Aux concentrations du produit, bien que non classé comme dangereux, l'azide de sodium peut réagir avec le cuivre et le plomb des canalisations et former des accumulations d'azides métalliques hautement explosives. Lors de l'élimination, rincer abondamment à l'eau pour éviter toute accumulation d'azide métallique dans les canalisations.
3. Comme avec tout produit d'origine biologique, des procédures de manipulation appropriées doivent être respectées.
4. Porter un équipement de protection individuelle approprié pour éviter tout contact avec les yeux et la peau.
5. Les solutions non utilisées doivent être éliminées conformément aux réglementations locales et nationales.

**Conservation**

Conserver entre 2 et 8 °C. Ne pas utiliser après la date de péremption imprimée sur le flacon. Si les réactifs sont conservés dans des conditions autres que celles indiquées, celles-ci doivent être validées par l'utilisateur. Aucun signe évident n'indique l'instabilité de ce produit. Par conséquent, des contrôles positifs et négatifs doivent être testés en même temps que les échantillons de patient. En cas de coloration inattendue, ne pouvant être expliquée par un changement des procédures du laboratoire, et de suspicion d'un problème avec l'anticorps, contacter l'assistance technique Dako.

**Guide rapide***

| Étape | | Commentaires |
|---|---|---|
| Fixation | Formol | |
| Prétraitement | EnVision FLEX™, High pH (réf. K8004) | HIER de 20 minutes, 3 en 1 avec PT Link et PT Link Rinse Station |
| Dilution | 1:50 | Incubation de 20 minutes |
| Tampon de dilution | Dako Antibody Diluent (réf. S0809) | Diluer immédiatement avant utilisation |
| Contrôle négatif | Dako Negative Control, Mouse IgG2a (réf. X0943) | Incubation de 20 minutes |
| Visualisation | EnVision™ FLEX, High pH (réf. K8000/K8010) | Incubation de 20 min, 2 incubations de 5 minutes avec le DAB+ |
| Contre-coloration | EnVision™ FLEX Hematoxylin (réf. K8008/K8018) | Incubation de 5 minutes |
| Tissu de contrôle | Amygdale, prostate | Coloration nucléaire |
| Lames | FLEX IHC Microscope Slides (réf. K8020) | Recommandées pour une meilleure adhérence des coupes de tissus aux lames de verre. |
| Montage | Milieu de montage permanent non aqueux requis | Une fois la procédure de coloration terminée, les coupes doivent être déshydratées, éclaircies et montées à l'aide d'un milieu de montage permanent. |
| Appareillage | Autostainer Link 48 et Autostainer Plus | Utiliser les flacons propres à l'instrument (réf. SK200-SK203 et S3425) |

**L'utilisateur doit toujours lire la notice pour obtenir des instructions détaillées sur la procédure de coloration et sur la manipulation du produit.*

**Préparation des échantillons**

Coupes en paraffine : L'anticorps peut être utilisé pour le marquage des coupes de tissus incluses en paraffine et fixées au formol. L'épaisseur des coupes d'échantillons tissulaires doit être d'environ 4 µm.

Prétraitement : Le prétraitement des coupes de tissus fixées au formol et incluses en paraffine par restauration d'épitope induit par la chaleur (HIER) est nécessaire. Des résultats optimaux sont obtenus en prétraitant les tissus par la méthode HIER à l'aide de la solution diluée EnVision™ FLEX Target Retrieval Solution, High pH (50x) (réf. K8004). Le déparaffinage, la réhydratation et la restauration d'épitope peuvent être réalisés sur l'appareil Dako PT Link (réf. PT100/PT101). Pour plus de détails, se référer au Guide d'utilisation du

PT Link. Les paramètres suivants doivent être utilisés pour le PT Link : température de préchauffage : 65 °C ; température et durée de la restauration d'épitope : 97 °C pendant 20 (±1) minutes ; laisser refroidir jusqu'à 65 °C. Retirer le portoir à lames de la cuve et plonger immédiatement les lames dans une jarre/cuve (ex. : PT Link Rinse Station, réf. PT109) contenant du EnVision™ FLEX Wash Buffer (20x) (réf. K8007) dilué à température ambiante. Laisser les lames dans le tampon de lavage pendant 1 à 5 minutes.

Les coupes de tissus ne doivent pas sécher lors du traitement ni lors de la procédure de coloration immunohistochimique qui suit. Pour une meilleure adhérence des coupes de tissus sur les lames de verre, il est recommandé d'utiliser les lames FLEX IHC Microscope Slides (réf. K8020). Après la coloration, les sections doivent être déshydratées, éclaircies et montées à l'aide d'un milieu de montage permanent.

**Procédure de coloration**

Dilution : La dilution recommandée pour l'anticorps Monoclonal Mouse Anti-Human p63 Protein, Clone DAK-p63, réf. M7317, est de 1:50. Diluer l'anticorps dans le diluant Dako Antibody Diluent (réf. S0809). Incuber les coupes de tissu prétraitées pendant 20 minutes à température ambiante. Il ne s'agit là que de conseils. Les conditions optimales peuvent varier en fonction de l'échantillon et de la méthode de préparation, et doivent être validées individuellement par chaque laboratoire.

Contrôle négatif : Le réactif de contrôle négatif recommandé est le Dako Negative Control, Mouse IgG2a (réf. X0943), dilué à la même concentration en IgG que l'anticorps primaire. À moins que la stabilité de l'anticorps dilué et du contrôle négatif n'ait été établie lors de la procédure de coloration en cours, diluer ces réactifs immédiatement avant utilisation. Les contrôles positifs et négatifs doivent être testés en même temps que les échantillons de patient.

Visualisation : Le système de visualisation recommandé est le système EnVision™ FLEX, High pH (réf. K8000/K8010) avec une durée d'incubation de 20 minutes, à température ambiante. Suivre la procédure incluse dans le ou les systèmes de visualisation choisis.

Automatisation : L'anticorps est bien adapté à la coloration immunohistochimique sur les plates-formes automatisées, telles que les systèmes Dako Autostainer, Autostainer Plus et Autostainer Link ainsi que le PT Link pour le prétraitement.

Contre-coloration : Le contre-colorant recommandé est le produit EnVision™ FLEX Hematoxylin (réf. K8008/K8018). Pour des résultats optimaux, l'utilisation d'un milieu de montage permanent non aqueux est recommandée.

Contrôles : Des tissus de contrôle positif et négatif doivent être testés en même temps et en suivant le même protocole que les échantillons de patient. Le tissu de contrôle positif doit comprendre l'amygdale et la prostate, et les cellules/structures doivent présenter des schémas de réaction tels que décrits pour ces tissus dans la section "Performances".

**Interprétation de la coloration**

Le motif de coloration cellulaire est nucléaire. Une coloration cytoplasmique dans les tissus anormaux a également été rapportée (12).

**Performances**

Tissus sains : Dans l'amygdale, les cellules épidermoïdes de l'épithélium présentent une coloration modérée à forte, tandis que dans la prostate, les cellules épidermoïdes basales présentent une coloration faible à modérée. Occasionnellement, un marquage cytoplasmique au niveau des granulocytes peut être observé.

| Type de tissu (nb. testés) | Éléments tissulaires positifs | Type de tissu (nb. testés) | Éléments tissulaires positifs |
|---|---|---|---|
| Surrénale (3) | 3/3 cellules surrénales (30%), coloration cytoplasmique | Ovaire (3) | 0/3 |
| Moelle osseuse (3) | 0/3 | Pancréas (3) | 3/3 cellules des îlots pancréatiques (100%), coloration cytoplasmique |
| Sein (3) | 3/3 cellules basales (90%), coloration nucléaire | Hypophyse (3) | 3/3 cellules adénohypophysaires (100%), coloration cytoplasmique |
| Cervelet (3) | 0/3 | Placenta (3) | 1/3 cellules syncytiotrophoblastiques (10%), coloration nucléaire |
| Cerveau (3) | 0/3 | Prostate (3) | 3/3 cellules basales (100%), coloration nucléaire |
| Col de l'utérus (3) | 3/3 cellules basales (100%), coloration nucléaire | Glande salivaire (3) | 3/3 cellules basales myoépithéliales (90%), coloration nucléaire |
| Côlon (3) | 0/3 | Peau (3) | 3/3 cellules épithéliales et cellules basales (100%), coloration nucléaire |
| Endomètre (3) | 0/3 | Intestin grêle (3) | 2/3 cellules épithéliales (10%), coloration cytoplasmique |
| Œsophage (3) | 3/3 cellules épithéliales (100%), coloration nucléaire | Moelle épinière (3) | 0/3 |
| Trompe de Fallope (3) | 3/3 cellules basales (50%), coloration nucléaire | Rate (3) | 0/3 |
| Rein (3) | 0/3 | Estomac (3) | 3/3 cellules glandulaires (30-100%), coloration cytoplasmique |
| Foie (3) | 0/3 | Testicule (3) | 0/3 |
| Poumon (3) | 1/3 cellules basales (100%), coloration nucléaire | Thyroïde (3) | 0/3 |
| Ganglion lymphatique (3) | 0/3 | Amygdale (3) | 3/3 cellules épithéliales (100%), coloration nucléaire |
| Muscle, cardiaque (3) | 0/3 | Utérus (3) | 2/3 cellules épithéliales (< 1%), coloration nucléaire |
| Muscle, squelettique (3) | 0/3 | Uretère (3) | 3/3 cellules épithéliales (100%), coloration nucléaire |
| Nerf périphérique (3) | 0/3 | Vessie (3) | 3/3 cellules épithéliales (100%), coloration nucléaire |

Tissus anormaux : L'anticorps a marqué les éléments suivants : cellules basales de 10/10 hyperplasies de la prostate et cellules myoépithéliales de 5/5 carcinomes du sein *in situ*, 6/6 carcinomes épidermoïdes pulmonaires, 6/6 carcinomes épidermoïdes du col utérin, 0/10 carcinomes de la prostate, 3/3 carcinomes du sein, 4/6 adénocarcinomes du col de l'utérus et 4/6 adénocarcinomes pulmonaires (14).

## DEUTSCH

**Verwendungszweck**

Zur In-vitro-Diagnostik.

Monoclonal Mouse Anti-Human p63 Protein, Clone DAK-p63, ist zur Verwendung in der Immunhistochemie bestimmt. Antikörper gegen Protein p63, das die Proliferation basaler Epithelzellen reguliert (1), können für die Erkennung von Adenokarzinomen der Prostata eingesetzt werden, wo sie als Hilfsmittel bei der Differenzierung zwischen gutartigen Prostataläsionen und Adenokarzinomen der Prostata dienen (2, 3). Antikörper gegen das Protein p63 können auch als Hilfsmittel bei der Differenzierung zwischen In-situ-Brustkarzinomen und Brustkarzinomen dienen (4), zur Unterscheidung eines Plattenepithelkarzinoms von einem Adenokarzinom der Lunge (5, 6) und zur Unterscheidung eines Plattenepithelkarzinoms der Zervix von einem Adenokarzinom der Zervix (7). Die klinische Auswertung einer eventuell eintretenden Färbung sollte durch morphologische Studien mit geeigneten Kontrollen ergänzt und von einem qualifizierten Pathologen unter Berücksichtigung der Krankengeschichte und anderer diagnostischer Tests des Patienten vorgenommen werden.

**Synonyme Bezeichnungen des Antigens**

Tumorprotein (p63).

**Zusammenfassung und Erklärung**

Das Protein p63 gehört ebenso wie p73 zur p53-Familie. Das p63-Gen kodiert für verschiedene Isoformen: Isoformen mit einer potenten aminoterminalen Transaktivierungsdomäne (TAp63-Isoformen) und Isoformen ohne diese Domäne (ΔNp63-Isoformen) (8, 9). Obwohl die TAp63-Isoformen p53-Zielgene, wie z. B. Bax und $p21^{WAF/CEP1}$ transaktivieren und eine Apoptose und einen Zellzyklusarrest induzieren können (10), handelt es sich bei p63 nicht um einen Tumorsuppressor (9). Die ΔNp63-Isoformen konkurrieren auf dominant-negative Weise um die p53-Zielgene und fördern indirekt das Zellwachstum, indem sie die Apoptose- und Zellzyklusarrest-Aktivierung durch TAp63-Isoformen und p53 konterkarieren (1, 10, 11).
P63 ist ein Marker für nicht-invasive epitheliale Tumoren, während bei invasiveren Tumoren ein Ausfall der p63-Expression festgestellt wird, was darauf hindeutet, dass ein Ausfall der p63-Expression die Entstehung von Tumoren und Metastasen beschleunigt (10). Das Fehlen von p63 ist jedoch kein zuverlässiger Marker für Invasivität, und obwohl p63 bei dem wenigsten Brustkarzinomen exprimiert wird, kommt es in seltenen Fällen zu einer nuklearen p63-Expression (9).
Häufig liegt bei Tumoren eine simultane transkriptionale Hochregulation der TAp63-Isoformen sowie der ΔNp63-Isoformen vor, wobei ΔNp63 auf der Proteinebene vorherrscht. Einige Lungenkarzinome und Plattenepithelkarzinome des Kopfs und Nackens weisen eine p63-Protein-Überexpression auf, verbunden mit einem leichten Anstieg der p63-Genkopienzahlen. Größtenteils handelt es sich bei p63-Isoformen jedoch um ΔNp63-Isoformen. Auch bei nasopharyngealen Karzinomen und Plattenepithelkarzinomen der Speiseröhre machen ΔNp63-Isoformen den Großteil der Isoformen aus (9).
Das p63-Protein kommt überwiegend in den Basalzellen von normalem Epithel in Ektozervix, Speiseröhre, Prostata, Haut, Mandeln, Urothel und Vagina sowie in Basalzellen in Drüsenstrukturen der Brust, Bronchien und Prostata vor. Daneben wird das p63-Protein in Myoepithelzellen der Brust exprimiert (9).

Folgende Angaben bitte den *General Instructions for Immunohistochemical Staining* (Allgemeine Richtlinien zur immunhistochemischen Färbung) von Dako oder den Anweisungen des Detektionssystems für IHC-Verfahren entnehmen:

**Geliefertes Reagenz**

Monoklonaler Maus-Antikörper in flüssiger Form als Zellkulturüberstand (mit fetalem Rinderserum), gegen 0,05 mol/L Tris-HCl, pH 7,2 und 0,015 mol/L Natriumazid dialysiert

Klon: DAK-p63. Isotyp: IgG2a, kappa.

Konzentration Maus-IgG mg/L: Siehe Behälteretikett.

Die Proteinkonzentration kann bei den Chargen verschieden ausfallen, ohne die optimale Verdünnung zu beeinflussen. Der Titer wird bei jedem einzelnen Los mit einem Referenzlos verglichen und diesem angeglichen, um konstante immunhistochemische Färbeergebnisse zwischen den Losen zu gewährleisten.

**Immunogen**

Synthetisches Peptid aus der DNA-bindenden Kerndomäne des humanen Tumorproteins p63

**Spezifität**

Beim Western-Blotting weist der Antikörper Banden nach, die dem jeweiligen erwarteten Molekulargewicht und Expressionsmuster der verschiedenen Isoformen (TAp63- und ΔNp63-Isoformen) von p63 bei einem HCC1806-Plattenepithelzellkarzinom-Lysat und bei Dickdarmkrebs entsprechen.

**Hinweise und Vorsichtsmaßnahmen**

1. Für geschultes Fachpersonal.
2. Dieses Produkt enthält Natriumazid ($NaN_3$), eine in reiner Form äußerst giftige Chemikalie. Ansammlungen von Natriumazid können auch in Konzentrationen, die nicht als gefährlich klassifiziert sind, mit Blei- und Kupferabflussrohren reagieren und hochexplosive Metallazide bilden. Nach der Entsorgung stets mit viel Wasser nachspülen, um Azidansammlungen in den Leitungen vorzubeugen.
3. Wie bei allen aus biologischen Materialien gewonnenen Produkten müssen die ordnungsgemäßen Handhabungsverfahren eingehalten werden.
4. Entsprechende Schutzkleidung tragen, um Augen- und Hautkontakt zu vermeiden.
5. Nicht verwendete Lösung ist entsprechend der örtlichen, staatlichen und EU-rechtlichen Bestimmungen zu entsorgen.

**Lagerung**

Bei 2 bis 8 °C aufbewahren. Nach Ablauf des auf dem Behälter aufgedruckten Verfallsdatums nicht mehr verwenden. Werden die Reagenzien nicht entsprechend den angegebenen Bedingungen aufbewahrt, müssen die Bedingungen vom Anwender geprüft werden. Es gibt keine offensichtlichen Anzeichen für eine eventuelle Produktinstabilität. Es sollten daher die Positiv- und Negativkontrollen gleichzeitig mit den Patientenproben mitgeführt werden. Falls eine unerwartete Färbung auftritt, die sich nicht durch Unterschiede bei Laborverfahren erklären lässt und auf ein Problem mit dem Antikörper hindeutet, ist der technische Kundendienst von Dako zu verständigen.

**Kurzanleitung***

| **Schritt** | | **Anmerkungen** |
|---|---|---|
| Fixierung | Formalin | |
| Vorbehandlung | EnVision FLEX™, High pH (Code-Nr. K8004) | 20 Min. HIER, 3-in-1 mit PT Link und PT Link Rinse Station |
| Verdünnung | 1:50 | 20 Min. Inkubation |
| Verdünnungspuffer | Dako Antibody Diluent (Code-Nr. S0809) | Unmittelbar vor Verwendung verdünnen |
| Negativkontrolle | Dako Negative Control, Mouse IgG2a (Code-Nr. X0943) | 20 Min. Inkubation |
| Detektionssystem | EnVision™ FLEX, High pH (Code-Nr. K8000/K8010) | 20 Min. Inkubation, 2 x 5 Min. DAB+ Inkubation |
| Gegenfärbung | EnVision™ FLEX Hematoxylin (Code-Nr. K8008/K8018) | 5 Min. Inkubation |
| Kontrollgewebe | Tonsille, Prostata | Nukleare Färbung |
| Objektträger | FLEX IHC Microscope Slides (Code-Nr. K8020) | Für eine bessere Haftung der Gewebeschnitte an den Glas-Objektträgern empfohlen. |

| | | |
|---|---|---|
| Eindecken | Nichtwässriges, permanentes Eindecken erforderlich | Nach dem Färben müssen die Schnitte dehydriert, geklärt und unter Verwendung eines permanenten Eindeckmediums auf Objektträger eingedeckt werden. |
| Geräte | Autostainer Link 48 und Autostainer Plus | Gerätespezifische Behälter verwenden (Code-Nr. SK200-SK203 und Code-Nr. S3425) |

**Der Anwender muss stets die Packungsbeilage lesen, um sich über die detaillierten Anweisungen für das Färbeverfahren und die Handhabung des Produkts zu informieren.*

**Probenvorbereitung**

Paraffinschnitte: Der Antikörper kann für die Markierung von formalinfixierten, paraffineingebetteten Gewebeschnitten verwendet werden. Gewebeproben sollten in Schnitte von ca. 4 µm Stärke geschnitten werden.

Vorbehandlung: Es ist eine Vorbehandlung der formalinfixierten und paraffineingebetteten Gewebeschnitte durch hitzeinduzierte Epitopdemaskierung (HIER) erforderlich. Optimale Ergebnisse können durch HIER-Vorbehandlung der Gewebe mit EnVision™ FLEX Target Retrieval Solution, High pH (50x) (Code-Nr. K8004) erzielt werden. Entparaffinierung, Rehydrierung und Epitopdemaskierung können in Dako PT Link (Code-Nr. PT100/PT101) durchgeführt werden. Weitere Informationen hierzu: siehe PT Link Benutzerhandbuch. Für PT Link sollten die folgenden Parameter verwendet werden: Vorwärmtemperatur: 65 °C; Temperatur und Zeit für Epitopdemaskierung: 20 (±1) Minuten bei 97 °C; auf 65 °C abkühlen. Das Objektträgergestell mit den Objektträgern aus dem Behälter herausnehmen und die Objektträger sofort in einen Behälter (z. B. PT Link Rinse Station, Code-Nr. PT109) mit verdünntem, auf Zimmertemperatur gebrachtem EnVision™ FLEX Wash Buffer (20x) (Code-Nr. K8007) eintauchen. Die Objektträger 1 bis 5 Minuten lang im Waschpuffer belassen.

Die Gewebeschnitte dürfen während der Behandlung oder des anschließenden immunhistochemischen Färbeverfahrens nicht austrocknen. Zur besseren Haftung der Gewebeschnitte an den Glasobjektträgern wird die Verwendung von FLEX IHC Microscope Slides (Code-Nr. K8020) empfohlen. Nach dem Färben müssen die Schnitte dehydriert, geklärt und unter Verwendung eines permanenten Eindeckmediums auf Objektträger eingedeckt werden.

**Färbeverfahren**

Verdünnung: Die empfohlene Verdünnung des Monoclonal Mouse Anti-Human p63 Protein, Clone DAK-p63, Code-Nr. M7317, ist 1:50. Den Antikörper in Dako Antibody Diluent (Code-Nr. S0809) verdünnen. Die vorbehandelten Gewebeschnitte 20 Minuten lang bei Raumtemperatur inkubieren. Diese Angaben sind nur Richtlinien. Optimale Bedingungen können je nach Probe und Vorbereitungsverfahren unterschiedlich sein und sollten vom jeweiligen Labor einzeln validiert werden.

Negativkontrolle: Als Negativkontrollreagenz wird Dako Negative Control, Mouse IgG2a (Code-Nr. X0943), empfohlen, das auf dieselbe Ig-Konzentration wie der primäre Antikörper verdünnt wurde. Falls die Stabilität des verdünnten Antikörpers und der Negativkontrolle für das verwendete Färbeverfahren nicht erwiesen ist, wird empfohlen, diese Reagenzien unmittelbar vor der Verwendung zu verdünnen. Positiv- und Negativkontrollen sollten zur gleichen Zeit wie die Patientengewebe getestet werden.
Detektionssystem: Das empfohlene Detektionssystem ist EnVision™ FLEX, High pH (Code-Nr. K8000/K8010) mit 20-minütiger Inkubation bei Raumtemperatur. Das für das ausgewählte Detektionssystem beschriebene Verfahren befolgen.

Automatisierung: Der Antikörper eignet sich sehr gut für immunhistochemische Färbungen mit automatisierten Systemen, z. B. mit Dako Autostainer, Autostainer Plus und Autostainer Link sowie PT Link für die Vorbehandlung.

Gegenfärbung: Als Gegenfärbung wird EnVision™ FLEX Hematoxylin (Code-Nr. K8008/K8018) empfohlen. Für optimale Ergebnisse wird ein nichtwässriges permanentes Eindeckmedium empfohlen.

Kontrollen: Positiv- und Negativkontrollgewebe sollten zur gleichen Zeit und mit demselben Protokoll wie die Patientengewebe getestet werden. Das Positivkontrollgewebe sollte Mandeln und Prostata enthalten und die Zellen/Strukturen müssen die unter „Leistungseigenschaften“ für dieses Gewebe beschriebenen Reaktionsmuster aufweisen.

**Auswertung der Färbung**

Das zelluläre Färbemuster ist nuklear. Eine zytoplasmatische Färbung bei anormalem Gewebe ist ebenfalls beschrieben worden (12).

**Leistungseigenschaften**

Normale Gewebe: In Mandelgewebe weisen die Plattenepithelzellen eine mäßige bis starke Färbereaktion auf. In Prostatagewebe weisen die Basalepithelzellen eine schwache bis mäßige Färbereaktion auf. Gelegentlich kann eine zytoplasmatische Markierung von Granulozyten auftreten.

| Gewebetyp (Anz. getestet) | Positive Gewebe-Elemente | Gewebetyp (Anz. getestet) | Positive Gewebe-Elemente |
|---|---|---|---|
| Nebenniere (3) | 3 von 3 Nebennierenzellen (30%), zytoplasmatisch | Eierstöcke (3) | 0 von 3 |
| Knochenmark (3) | 0 von 3 | Pankreas (3) | 3 von 3 Inselzellen (100%), zytoplasmatisch |
| Brust (3) | 3 von 3 Basalzellen (90%), nuklear | Hypophyse (3) | 3 von 3 Hypophysenzellen (100%), zytoplasmatisch |
| Zerebellum (3) | 0 von 3 | Plazenta (3) | 1 von 3 Synzytiotrophoblastzellen (<10 %), nuklear |
| Zerebrum (3) | 0 von 3 | Prostata (3) | 3 von 3 Basalzellen (100%), nuklear |
| Zervix (3) | 3 von 3 Basalzellen (100%), nuklear | Speicheldrüse (3) | 3 von 3 Myoepithelzellen (90%), nuklear |
| Dickdarm (3) | 0 von 3 | Haut (3) | 3 von 3 Epithelzellen und Basalzellen (100%), nuklear |
| Endometrium (3) | 0 von 3 | Dünndarm (3) | 2 von 3 Epithelzellen (10%), zytoplasmatisch |
| Speiseröhre (3) | 3 von 3 Epithelzellen (100 %), nuklear | Rückenmark (3) | 0 von 3 |
| Eileiter (3) | 3 von 3 Basalzellen (50%), nuklear | Milz (3) | 0 von 3 |
| Niere (3) | 0 von 3 | Magen (3) | 3/3 Drüsenzellen (30-100%), zytoplasmatisch |
| Leber (3) | 0 von 3 | Hoden (3) | 0 von 3 |
| Lunge (3) | 1 von 3 Basalzellen (100%), nuklear | Schilddrüse (3) | 0 von 3 |
| Lymphknoten (3) | 0 von 3 | Tonsille (3) | 3 von 3 Epithelzellen (100%), nuklear |
| Herzmuskel (3) | 0 von 3 | Uterus (3) | 2 von 3 Epithelzellen (<1%), nuklear |
| Skelettmuskulatur (3) | 0 von 3 | Ureter (3) | 3 von 3 Epithelzellen (100%), nuklear |
| Nerv, peripher (3) | 0 von 3 | Harnblase (3) | 3 von 3 Epithelzellen (100 %), nuklear |

Anormale Gewebe: Der Antikörper markierte Basalzellen bei 10 von 10 Prostatahyperplasien und Myoepithelzellen bei 5 von 5 In-situ-Brustkarzinomen. Der Antikörper markierte 6 von 6 Plattenepithelkarzinomen der Lunge, 6 von 6 Plattenepithellkarzinomen der Gebärmutterzervix, 0 von 10 Prostatakarzinomen, 3 von 3 Brustkarzinomen, 4 von 6 Adenokarzinomen der Zervix und 4 von 6 Adenokarzinomen der Lunge (14).

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**Explanation of symbols / Explication des symboles / Erläuterung der Symbole**

| | | |
|---|---|---|
| REF<br>Catalogue number<br>Référence du catalogue<br>Katalognummer | 2 °C – 8 °C<br>Temperature limitation<br>Limites de température<br>Zulässiger Temperaturbereich | Use by<br>Utiliser avant<br>Verwendbar bis |
| IVD<br>In vitro diagnostic medical device<br>Dispositif médical de diagnostic in vitro<br>In-vitro-Diagnostikum | LOT<br>Batch code<br>Réf. du lot<br>Chargenbezeichnung | Manufacturer<br>Fabricant<br>Hersteller |
| Consult instructions for use<br>Consulter les instructions d'utilisation<br>Gebrauchsanweisung beachten | EC REP<br>Authorized representative in the European Community<br>Représentant agréé dans la Communauté européenne<br>Autorisierte Vertretung in der Europäischen Gemeinschaft | |


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# Assessment Run 58 2020

# Pan Cytokeratin (CK-PAN)

**Purpose**
Evaluation of the technical performance, level of analytical sensitivity and specificity of IHC tests among the NordiQC participants for CK-PAN used to identify the epithelial origin of carcinoma of unknown primary origin. Relevant clinical tissues, both normal and neoplastic, were selected to include a wide spectrum of CK-PAN antigen densities (see below).

**Material**
The slide to be stained for CK-PAN comprised:


1. Appendix, 2. Liver, 3. Esophagus, 4. Tonsil, 5. Lung adenocarcinoma,
6. Lung squamous cell carcinoma, 7. Renal clear cell carcinoma (CCRCC),
8. Diffuse large B-cell lymphoma (DLBCL).

Criteria for assessing a CK-PAN staining as optimal were:

- A strong, distinct cytoplasmic staining reaction of all bile ductal epithelial cells and an at least moderate cytoplasmic staining reaction with membrane accentuation of the majority of hepatocytes.
- A strong, distinct cytoplasmic staining reaction of all squamous epithelial cells throughout all cell layers in the esophagus and tonsil.
- A strong, distinct cytoplasmic staining reaction of virtually all neoplastic cells in the lung adenocarcinoma and squamous cell carcinoma.
- An at least weak to moderate, predominantly membranous staining reaction of the majority of neoplastic cells in the renal clear cell carcinoma.
- No staining in lymphocytes in tonsil and neoplastic cells in the DLBCL. Interstitial reticulum cells (CIRCs) with dendritic/reticular pattern was accepted and expected to show a weak to moderate cytoplasmic staining reaction due to expression of cytokeratin low mol. weight types 8/18.

All tissues were fixed in 10% neutral buffered formalin.

**Participation**

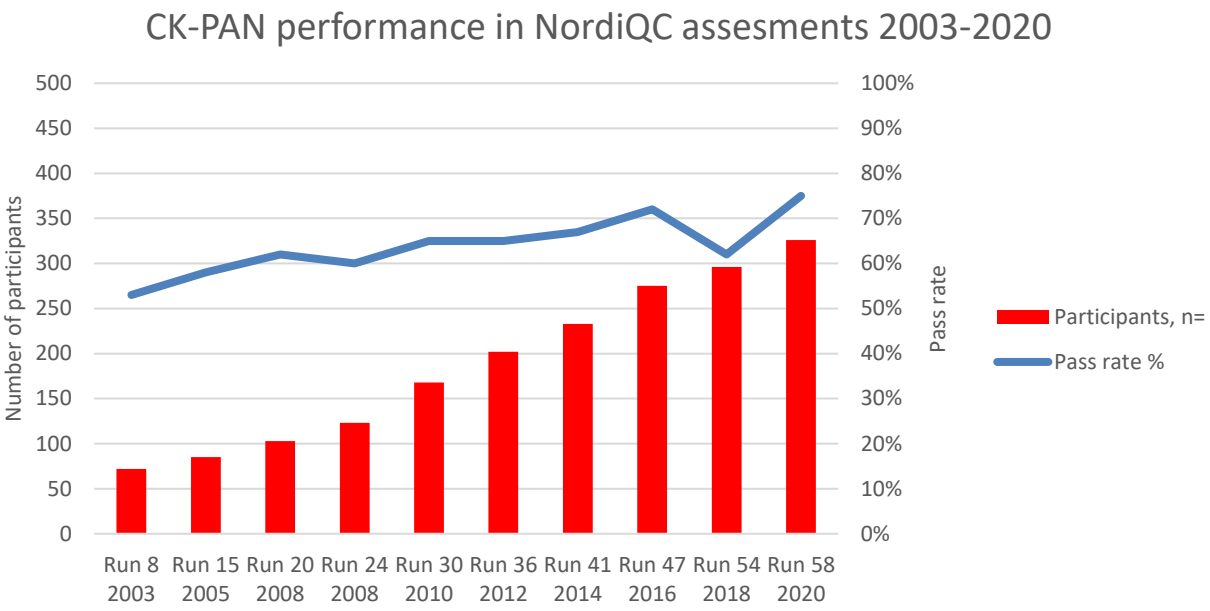
| | |
|---|---|
| Number of laboratories registered for CK-PAN, run 58 | 342 |
| Number of laboratories returning slides | 326 (95%) |

**Results**
326 laboratories participated in this assessment. 243 (75%) achieved a sufficient mark (optimal or good). Table 1 summarizes the antibodies (Abs) used and assessment marks (see page 2).

The most frequent causes of insufficient staining were:

- Too low concentration of the primary antibody
- Insufficient HIER – too short efficient heating time and/or use of non-alkaline HIER buffers
- Inappropriate epitope retrieval
- Less successful primary antibodies, especially mAb clone MNF116
- Less successful performance of the mAb clone cocktail AE1/AE3 on the Leica Bond platforms
- Technical issues.

**Performance history**
This was the tenth NordiQC assessment of CK-PAN. The overall pass rate increased significantly compared to the previous run 54, see Graph 1.




Graph 1. **Proportion of sufficient results for CK-PAN in the ten NordiQC run performed**

**Conclusion**

The mAb clone cocktails **AE1/AE3, AE1/AE3/PCK26** and mAb clone **BS5** can all be recommended for demonstration of CK-PAN. The mAb clone MNF116 should not be used due to a general poor performance. The epitope retrieval method must be specifically tailored to the clone/cocktail applied. The mAb clone cocktail AE1/AE3 showed an inferior performance on the Bond platform (Leica), and used within a laboratory developed assay, no optimal results could be obtained, whereas mAb clone BS5 was found to be more successful on the Bond platform. The Ready-To-Use (RTU) systems from Dako based on mAb clone cocktail AE1/AE3 were in this assessment the most successful and provided high proportions of sufficient and optimal results.

Liver and tonsil/esophagus in combination are recommendable as positive and negative tissue controls. The vast majority of hepatocytes must show a distinct cytoplasmic staining reaction with membrane accentuation, while virtually all squamous epithelial cells of the tonsil/esophagus throughout all cell layers must display a strong cytoplasmic staining reaction. Tonsil can also be used as negative tissue control, in which no staining reaction should be seen in lymphocytes. Dispersed interstitial reticulum cells with dendritic/reticular pattern can show a weak to moderate cytoplasmic staining reaction and must be accepted due to low level CK expression.

Table 1. **Antibodies and assessment marks for CK-PAN, run 58**

| Concentrated antibodies | n | Vendor | Optimal | Good | Borderline | Poor | Suff.[1] | OR[2] |
|---|---|---|---|---|---|---|---|---|
| mAb clone cocktail **AE1/AE3** | 73<br>2<br>10<br>10<br>2<br>1<br>1<br>1<br>1<br>1<br>1<br>1 | Dako/Agilent<br>Thermo/NeoMarkers<br>Cell Marque<br>Leica/Novocastra<br>Biocare Medical<br>Zytomed<br>Diagnostic Biosystems<br>Genemed<br>Immunologic<br>DCS Diagnostics<br>Bio Sp<br>Zytomed<br>Zeta Corporation | 51 | 26 | 18 | 9 | 74% | 49% |
| mAb clone cocktail **AE1/AE3/5D3** | 2<br>1<br>1 | Biocare Medical<br>Zytomed<br>Abcam | - | 3 | - | 1 | - | - |
| mAb clone cocktail **PAN CK (Ab C2562)** | 1 | Sigma Aldrich | - | 1 | - | - | - | - |
| mAb clone **BS5** | 4<br>10 | Monosan<br>Nordic Biosite | 8 | 4 | 2 | - | 86% | 57% |
| mAb clone **MNF116** | 9 | Dako/Agilent | - | - | - | 9 | 0% | 0% |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| mAb clone **OSCAR** | 1 | PhenoPath | - | - | 1 | - | - | - |
| mAb clone **KL1** | 1 | Zytomed | - | - | - | 1 | - | - |
| "Laboratory made" antibody cocktails | | | | | | | Suff.[1] | OR.[2] |
| Ab clone cocktail **AE1/AE3/8/18** | 1 | Leica/Novocastra | 1 | - | - | - | - | - |
| mAb clone cocktail **Unknown** | 2 | | 1 | 1 | - | - | - | - |
| Ready-To-Use antibodies | | | | | | | Suff.[1] | OR.[2] |
| mAb clone cocktail **AE1/AE3 IR053 (VRPS)[3]** | 13 | Dako/Agilent | 12 | - | - | 1 | 92% | 92% |
| mAb clone cocktail **AE1/AE3 IR053 (LMPS)[4]** | 14 | Dako/Agilent | 10 | 2 | 2 | - | 86% | 71% |
| mAb clone cocktail **AE1/AE3 GA053 (VRPS)[3]** | 31 | Dako/Agilent | 27 | 1 | 2 | 1 | 90% | 87% |
| mAb clone cocktail **AE1/AE3 GA053 (LMPS)[4]** | 18 | Dako/Agilent | 17 | 1 | - | - | 100% | 94 |
| mAb clone cocktail **AE1/AE3 313M-XX** | 2 | Cell Marque | - | 1 | 1 | - | - | - |
| mAb clone cocktail **AE1/AE3 MAD 001000QD** | 1 | Master Diagnostica | - | 1 | - | - | - | - |
| mAb clone cocktail **AE1/AE3 PA0909** | 2 | Leica/Novocastra | - | 1 | 1 | - | - | - |
| mAb clone cocktail **AE1/AE3 PA0094** | 5 | Leica/Novocastra | 1 | 3 | 1 | - | 80% | 20% |
| mAb clone cocktail **AE1/AE3 PA0012** | 3 | Leica/Novocastra | - | 3 | - | - | - | - |
| mAb clone cocktail **AE1/AE3 PDM072** | 2 | Diagnostic Biosystems | - | - | 2 | - | - | - |
| mAb clone cocktail **AE1/AE3/PCK26 760-2135/2595 (VRPS)[3]** | 25 | Ventana/Roche | 11 | 8 | 4 | 2 | 76% | 44% |
| mAb clone cocktail **AE1/AE3/PCK26 760-2135/2595 (LMPS)[4]** | 69 | Ventana/Roche | 29 | 19 | 10 | 11 | 70% | 42% |
| mAb clone cocktail **AE1/AE3/ VP011** | 1 | Biocare Medical | - | - | 1 | - | - | - |
| mAb clone **Lu-5 AM181-5M** | 1 | Biogenex | - | - | - | 1 | - | - |
| mAb clone cocktail **AE1/AE3/DC10 8309-C010** | 1 | Sakura Finetek | 1 | - | - | - | - | - |
| mAb clone **OSCAR Z-465-26-Y** | 1 | Zytomed Systems | - | - | 1 | - | - | - |
| Total | 326 | | 168 | 75 | 47 | 36 | - | |
| Proportion | | | 52% | 23% | 15% | 11% | 75% | |



1) Proportion of sufficient stains (optimal or good). (≥5 asessed protocols)
2) Proportion of Optimal Results (≥5 asessed protocols).
3) Vendor Recommended Protocol Settings (VRPS) to a specific RTU product applied on the vendor recommended platform(s) (≥5 asessed protocols).
4) Laboratory Modified Protocol Settings (LMPS) to a specific RTU product (≥5 asessed protocols).

**Detailed analysis of CK-PAN, Run 58**
The following protocol parameters were central to obtain optimal staining:

**Concentrated antibodies**
mAb clone cocktail **AE1/AE3**: Protocols with optimal results were all based on Heat Induced Epitope Retrieval (HIER) using Target Retrieval Solution (TRS) pH 9 (3-in-1) (Dako) (11/15)*, Cell Conditioning 1 (CC1, Ventana) (36/62) or Tris-EDTA/EGTA pH 9 (3/3) as retrieval buffer. The mAb was typically diluted in the range of 1:50-1:200 depending on the total sensitivity of the protocol employed. Using these protocol settings, 63 of 70 (90%) laboratories produced a sufficient staining result (optimal or good).
* (number of optimal results/number of laboratories using this HIER buffer)

mAb clone **BS5**: Protocols with optimal results were based on HIER using TRS pH 9 (Dako) (1/3), Tris/EDTA pH 9 (1/1), CC1 (Ventana) (2/3), Bond Epitope Retrieval Solution 2 (BERS2, Leica) 3/6 and Bond Epitope Retrieval Solution 1 (BERS1, Leica) 1/1.The mAb was diluted in the range of 1:100-1:800 depending on the total sensitivity of the protocol employed. Using these settings 12/14 (86%) laboratories produced a sufficient staining result. One of the protocols that obtained an insufficient mark was due to technical issues.

Table 2. **Proportion of optimal results for CK-PAN using the mAb clone cocktail AE1/AE3 as concentrate on the four main IHC systems***

| Concentrated antibodies | **Dako/Agilent Autostainer** | | **Dako/Agilent Omnis** | | **Ventana/Roche BenchMark XT / Ultra** | | **Leica Bond III / Max** | |
|---|---|---|---|---|---|---|---|---|
| | TRS pH 9.0 | TRS pH 6.1 | TRS pH 9.0 | TRS pH 6.1 | CC1 pH 8.5 | CC2 pH 6.0 | BERS2 pH 9.0 | BERS1 pH 6.0 |
| mAb clone **AE1/AE3** | 5/9** (56%) | - | 6/6 100% | - | 36/62 (58%) | - | 0/12 (0%) | 0/3 |
| mAb clone **BS5** | 0/2 | - | 1/1 | - | 2/3 | - | 3/6 | 1/1 |

* Antibody concentration applied as listed above, HIER buffers and detection kits used as provided by the vendors of the respective systems.
** Number of optimal results/number of laboratories using this buffer.

**Ready-To-Use antibodies and corresponding systems**
mAb clone cocktail **AE1/AE3**, product no. **IR053**, Dako, Autostainer+/Autostainer Link:
Protocols with optimal results were typically based on HIER in PT-Link using TRS pH 9 (3-in-1) (efficient heating time 10-40 min. at 95-97°C), 15-30 min. incubation of the primary Ab and EnVision FLEX (K8000) as detection system. Using these protocol settings, 20 of 21 (95%) laboratories produced a sufficient staining result. One laboratory did not produce a sufficient result with these settings due to technical issues.

mAb clone cocktail **AE1/AE3**, product no. **GA053**, Dako, OMNIS:
Protocols with optimal results were based on HIER using TRS pH 9 (3-in-1) (efficient heating time 20-30 min. at 97°C) and 10-20 min. incubation of the primary Ab and EnVision FLEX (GV800/GV823) as detection system. Using these protocol settings, 40 of 43 (93%) laboratories produced a sufficient staining result. Two of the protocols obtaining an insufficient mark was due to technical issues.

mAb clone cocktail **AE1/AE3/PCK26,** product no. **760-2135/2595**, Ventana, BenchMark GX/XT/Ultra:
Protocols with optimal results were typically based on a combined pre-treatment using HIER in CC1 for 24-64 min. followed by enzymatic pre-treatment in Protease 3 (4 min.), 4-24 min. incubation of the primary Ab and UltraView with or without amplification (760-500+760-080) or OptiView (760-700) as detection system. Using these protocol settings, 42 of 48 (88%) laboratories produced a sufficient staining result. Four of the protocols obtaining an insufficient mark due to technical issues.

Table 3 summarizes the proportion of sufficient and optimal marks for the most commonly used RTU systems (≥10 asessed protocols). The performance was evaluated both as "true" plug-and-play systems performed strictly accordingly to the vendor recommendations and by laboratory modified systems changing basal protocol settings. Only protocols performed on the intended IHC stainer device are included.

Table 3. **Proportion of sufficient and optimal results for CK-PAN in the most commonly used RTU IHC systems**

| RTU systems | **Recommended protocol settings*** | | **Laboratory modified protocol settings**** | |
|---|---|---|---|---|
| | Sufficient | Optimal | Sufficient | Optimal |
| Dako AS<br>mAb AE1/AE3<br>**IR053** | 92% (12/13) | 92% (12/13) | 100% (9/9) | 78% (7/9) |
| Dako Omnis<br>mAb AE1/AE3<br>**GA053** | 90% (28/31) | 87% (27/31) | 100% (16/16) | 100% (16/16) |
| VMS Ultra/XT/GX<br>mAb AE1/AE3/PCK26<br>**760-2135/2595** | 76% (19/25) | 44% (11/25) | 70% (48/69) | 42% (29/69) |

* Protocol settings recommended by vendor – Retrieval method and duration, Ab incubation times, detection kit, IHC stainer/equipment.
** Modifications included: retrieval method, retrieval duration, retrieval reagents, Ab incubation time and detection kit. Only protocols performed on the specified vendor IHC stainer were included.

**Comments**

In concordance with the previous NordiQC assessments for CK-PAN, the prevalent feature of an insufficient staining result was a too weak or completely false negative staining reaction of cells and structures expected to be demonstrated. Virtually all participating laboratories were able to stain cytokeratins (CK) in the epithelial cells of bile ducts in liver and neoplastic cells of the lung adenocarcinoma, whereas demonstration of CK in hepatocytes and the neoplastic cells of the renal clear cell carcinoma was more difficult and was only obtained by protocols with appropriate protocol settings. The pass rate was highly influenced by the choice of Ab and retrieval method applied, which underlines the necessity for individual optimization for each clone/clone cocktail used for the demonstration of CK. This correlation, observed in the last nine NordiQC CK-PAN assessments, is summarized in Table 4.

Table 4. **Pass rates for antibody cocktails combined with epitope retrieval methods in nine NordiQC runs**

| **Pass rate for compiled data from run 15, 20, 24, 30, 36, 41, 47, 54 & 58** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Total** | | **HIER** | | **Proteolysis** | | **HIER + proteolysis** | |
| | Protocols | Sufficient | Protocols | Sufficient | Protocols | Sufficient | Protocols | Sufficient |
| **mAb AE1/AE3** | 1145 | 836 (73%) | 1075 | 826 (77%) | 49 | 6 (12%) | 9 | 3 (33%) |
| **mAb AE1/AE3/5D3** | 48 | 42 (88%) | 47 | 42 (89%) | 1 | 0 | 0 | 0 |
| **mAb AE1/AE3/PCK26** | 361 | 219 (61%) | 48 | 22 (46%) | 48 | 3 (6%) | 258 | 192 (74%) |
| **mAb MNF116** | 111 | 31 (28%) | 53 | 9 (17%) | 48 | 22 (46%) | 9 | 2 (22%) |

The data clearly stresses that the choice of epitope retrieval has significant impact on the staining result. For the most widely used Ab clone cocktail AE1/AE3, the overall pass rate in these 9 successive NordiQC runs was 73%. Using HIER, a pass rate of 77% was obtained, significantly higher than the pass rate of 12% when proteolytic pre-treatment was applied for AE1/AE3. For the second most commonly used Ab clone cocktail, AE1/AE3/PCK26, combined epitope retrieval using HIER in CC1 (Ventana) followed by proteolysis, provided a pass rate of 74%, compared to 46% and 6% using either HIER or proteolysis as single retrieval method.
The mAb clone MNF116 has in these nine consecutive runs provided an inferior overall performance compared to the 3 other antibody cocktails listed in Table 4. No significant improvement of the performance could be identified by any of the different retrieval methods. Consequently, mAb clone MNF116 should be substituted by e.g. one of the mentioned Ab cocktails or the mAb clone BS5.

42% (136 of 326) of the participants used a laboratory developed (LD) assay and the mAb clone cocktail AE1/AE3 and the mAb clone BS5 could both be used to obtain an optimal staining result for CK-PAN (see Table 1). For both clones used, HIER in an alkaline buffer was mandatory for optimal performance.
The mAb clone cocktail AE1/AE3 was the most widely used antibody for demonstration of CK-PAN and used as a concentrate, mAb clone cocktail AE1/AE1 gave an overall pass rate of 74% (77 of 104). As shown in Table 2, the performance of mAb clone cocktail AE1/AE3 seems to be influenced by the IHC stainer platform as optimal results could only be obtained on the platforms from Dako and Ventana, providing a careful calibration of the primary Ab and selection of appropriate protocol settings (Figs. 5-6). For yet unexplained reasons the mAb AE1/AE3 showed an overall inferior performance on the Bond platforms (Leica) where only 19% (3 of 16) were assessed as sufficient, none of which being optimal. No single parameter could be identified as root cause for the inferior performance

As mentioned in assessment report for run 54 (2018), too weak or false negative staining result was the main feature of an insufficient result and was typically caused by protocols with too low sensitivity. The titer of the primary Ab must be carefully calibrated to provide an IHC protocol, which is "fit-for-the-purpose", i.e. a protocol able to demonstrate CK-PAN in structures with both low-level and high-level CK expression, which is the range seen in carcinomas.

Although the number of participants using the mAb clone BS5 within a LD assay was low, this primary Ab seems robust and promising, as most protocols (12 of 14) were assessed as sufficient (see Table 1). This Ab might be an alternative to the more challenging Abs (e.g. MNF116 or AE1/AE3) on the Bond (Leica) platforms where 6/7 received a sufficient mark using mAb BS5. The mAb BS5 performed optimally using a dilution in the range of 1:100-1:800 carefully calibrated according to the overall sensitivity of the detection systems applied and using HIER in an alkaline buffer (e.g. BERS2, Leica) (Figs. 7a-b).

58% (190 of 326) of the laboratories used a Ready-To-Use (RTU) format for detection of CK-PAN. The number of assays based on these RTU formats is consistently increasing (compare with previous runs for CK-PAN on the NordiQC webpage). Ideally, a RTU format of a primary Ab should be used within a system that has been thoroughly validated, providing precise information on vendor recommended protocol settings, equipment, reagents and performance characteristics (expected reaction patterns). With this in focus, NordiQC has expanded the data analysis for the RTU systems from the main providers to assess the pass rates and proportion of optimal results when these systems are applied as "plug-and-play" or used with protocol modifications by the laboratories. The data can be seen in both Table 1 and Table 3.

In this assessment, the Dako RTU systems **IR053, IS053** and **GA053** based on the mAb clone cocktail AE1/AE3 provided the highest number of sufficient and optimal results. As shown in Table 3, and for laboratories using one of these systems, vendor recommended protocol settings gave a pass rate of 91% (40 of 44) of which 89% were assessed as optimal. Laboratory modified protocol settings (typically adjusting HIER and incubation time of the primary Ab) also provided high proportion of sufficient and optimal results.

The Ventana RTU system **760-2135/2595** was used by 94 participants and typically by laboratory modified protocol settings as shown in Table 1 and 3. When the RTU system was used by the vendor recommended protocol settings primarily based on a combined pre-treatment with HIER in CC1 and proteolysis in P3, a pass rate of 76% was observed, 44% being optimal. In general the pass rate and proportion of optimal results was reduced for laboratories modifying the protocol settings. Less successful modifications were especially related to e.g. substitution of P3 with P1 or P2, or use of proteolysis as single retrieval method. These specific modifications provided an overall inferior pass rate of 42%, 16% optimal. Typical staining patterns of these different pre-treatment procedures are illustrated in Figs. 1-4.

This was the tenth assessment of CK-PAN in NordiQC (see Graph 1). Although CK-PAN has been used for many years and is a central part of the primary panel for the identification and classification of carcinoma of unknown primary origin (together with S100, Vimentin and CD45), the marker is still technically challenging although the pass rate in this run 58 increased compared to the latest run 54, 2018. Several elements influenced the final outcome:

1) Less successful performance on the Bond platform (Leica) which is one of the 4 main IHC platforms. 42 participants used this platform and 48% received a sufficient mark, only 14% with optimal results.
2) 94 participants used the RTU system 760-2135/2595 (Ventana) and only 27% of them followed the vendor recommendation with combined pretreatment with HIER in CC1 and proteolysis in P3. Laboratory based modifications substituting P3 with P1 or 2, or using single retrieval methods, caused a decrease of the pass-rate to 42%.
3) The use of the less successful clone MNF116.

Conclusive and of central importance, laboratories should apply an Ab that will work on their in-house IHC platform, calibrate the protocols correctly with focus on appropriate settings for the specific clone and verify the protocol according to the expected antigen level and pattern in the recommended tissue control materials (see below).

**Controls**

As seen in the previous NordiQC assessments, liver and esophagus in combination are recommendable as positive tissue controls for CK-PAN. It is crucial that the vast majority of hepatocytes (expressing only a limited amount of the primary LMW-CK types 8 and 18) show an at least moderate, distinct cytoplasmic and membranous staining reaction. No staining should be seen in stromal cells in the liver. In esophagus, virtually all squamous epithelial cells throughout all cell layers must show a strong distinct cytoplasmic staining reaction due to expression of HMW-CK types 5 and 14. Smooth muscle cells in vessels and in muscularis mucosae in esophagus will typically show a weak to moderate patchy cytoplasmic staining

reaction. As alternative to esophagus and concordant to the guidelines published by the International Ad Hoc Expert Committee[1] for positive tissue controls, tonsil can be used as positive tissue control but also as a negative tissue control. If used as negative tissue control, no staining reaction should be seen in lymphocytes, whereas dispersed interstitial reticulum cells with dendritic/reticular pattern can show a weak to moderate cytoplasmic staining reaction and must be accepted due to low level CK expression.

[1]Torlakovic EE, Nielsen S, Francis G, Garratt J, Gilks B, Goldsmith JD, Hornick JL, Hyjek E, Ibrahim M, Miller K, Petcu E, Swanson PE, Zhou X, Taylor CR, Vyberg M. Standardization of positive controls in diagnostic immunohistochemistry: recommendations from the International Ad Hoc Expert Committee. Appl Immunohistochem Mol Morphol. 2015 Jan;23(1):1-18. doi: 10.1097/PAI.0000000000000163. Review. PubMed PMID: 25474126.

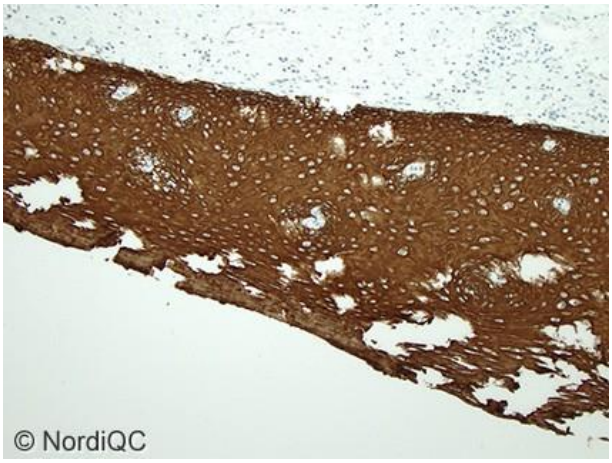



Fig. 1a (x100)
Optimal CK-PAN staining of the esophagus using the mAb clone cocktail AE1/AE3/PCK26 as RTU (Ventana), combined retrieval using HIER in CC1 (32 min.), proteolysis P3 (4 min.) and a 3-step multimer based detection system (OptiView).
All squamous epithelial cells show a strong and distinct cytoplasmic staining reaction.
Same protocol used in Figs. 2a-4a.

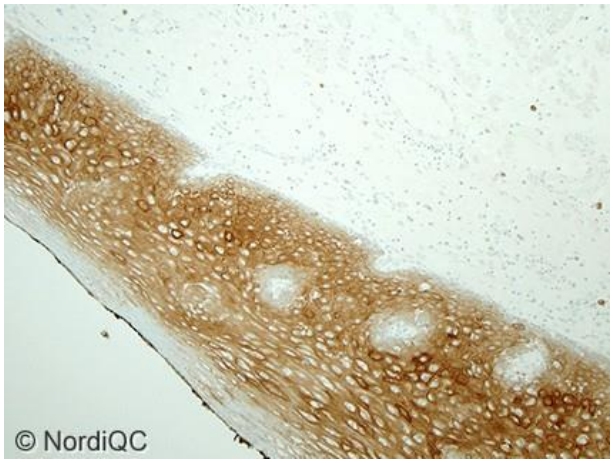



Fig. 1b (x100)
CK-PAN staining of the esophagus using an insufficient protocol with too low sensitivity based on the mAb clone cocktail AE1/AE3/PCK26 RTU (Ventana), but only with proteolysis in P1 as pre-treatment and UltraView as the detection system.
Same protocol used in Figs. 2b-4b.
The squamous epithelial cells show only a weak to moderate cytoplasmic staining reaction and are completely negative in the basal and upper layers of the squamous epithelium - compare to Fig 1.a (same field).

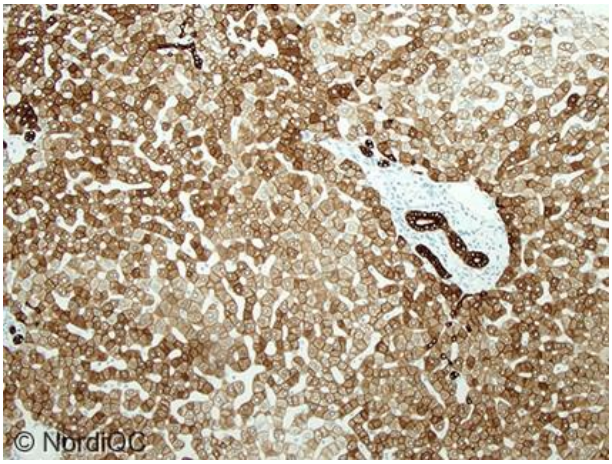



Fig. 2a (x100)
Optimal CK-PAN staining of the liver using same protocol as in Fig. 1a. The vast majority of hepatocytes show a moderate staining reaction (with membranous accentuation) while the columnar cells of the bile ducts display a strong cytoplasmic staining reaction.

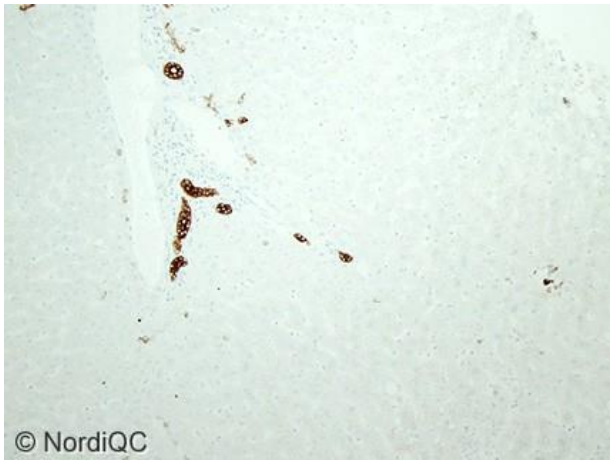



Fig. 2b (x100)
Insufficient CK-PAN staining of the liver using same protocol as in Fig. 1b.
Only epithelial cells of bile ducts are demonstrated due to high expression levels of CK-LMW (CK types 7, 8/18 and 19) whereas the hepatocytes are false negative (only express low antigen levels of CK 8/18).

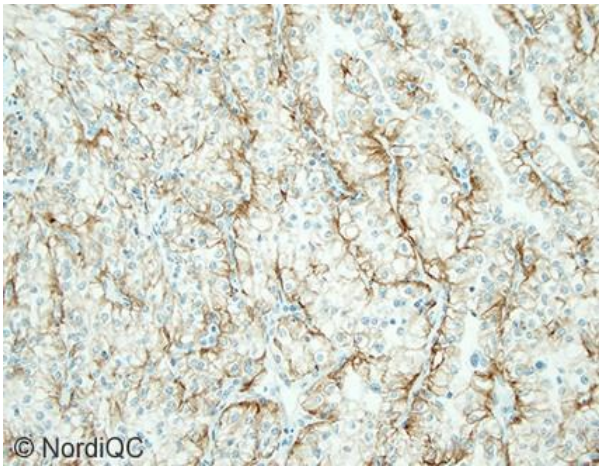



Fig. 3a (x200)
Optimal CK-PAN staining of the CCRCC using same protocol as in Figs. 1a and 2a. The vast majority of neoplastic cells display a weak to moderate, distinct cytoplasmic staining reaction with membranous accentuation.

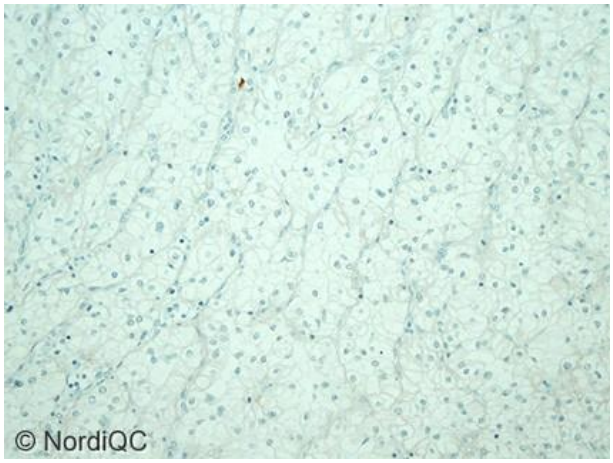



Fig. 3b (x200)
Insufficient CK-PAN staining of the CCRCC using same protocol as in Figs. 1b and 2b. The neoplastic cells are almost completely negative - same field as in Fig. 3a.

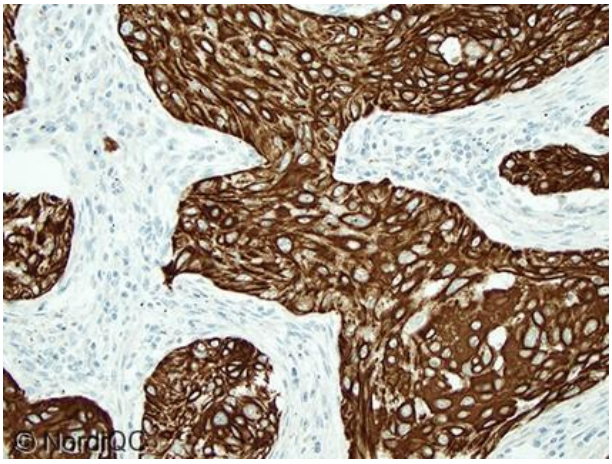



Fig. 4a (x200)
Optimal CK-PAN staining of the lung squamous cell carcinoma using same protocol as in Figs. 1a-3a. Virtually all neoplastic cells show a moderate to strong and distinct cytoplasmic staining reaction.

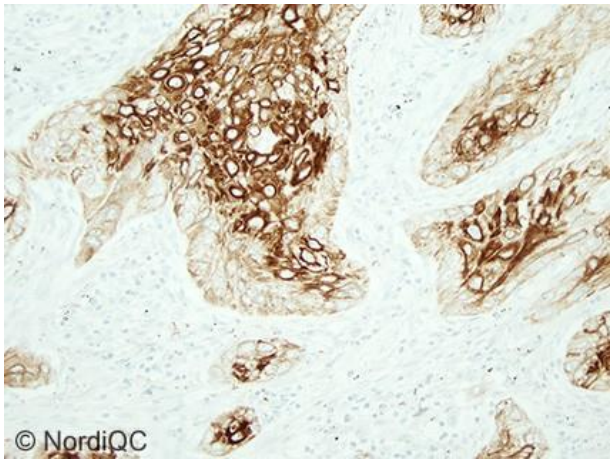



Fig. 4b (x200)
CK-PAN staining of the lung squamous cell carcinoma using same protocol as in Figs. 1b-3b – same field as in Fig. 4a. Virtually all squamous epithelial cells are demonstrated, but the intensity is reduced compared to the level expected (also compare with Fig. 3b, same protocol).

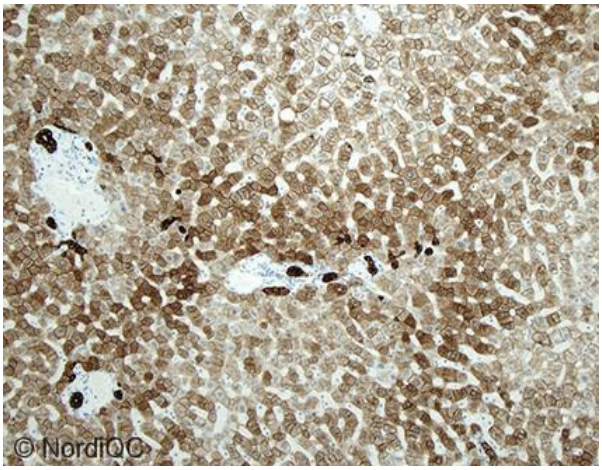



Fig. 5a (x100)
Optimal CK-PAN staining of the liver using the concentrated format of mAb clone cocktail AE1/AE3 (Dako/Agilent) on a Dako Autostainer, diluted 1:100 (20 min.), HIER in TRS High (10 min.) and a 3-step polymer based detection system (Envision Flex+, Dako). Same protocol for Fig. 6a.

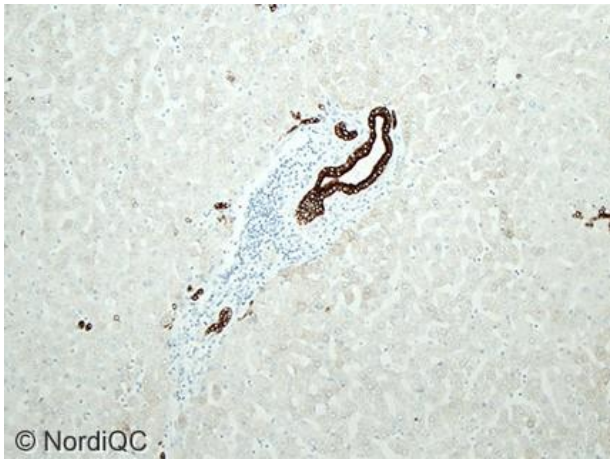



Fig. 5b (x100)
Insufficient CK-PAN staining of the liver using the concentrated format of mAb clone cocktail AE1/AE3 (Dako/Agilent) on a Ventana BenchMark Ultra, diluted, 1:100 (32 min.), HIER in CC1 (36 min.) and a 2-step multimer based detection system (UltraView, Ventana). The hepatocytes are only weakly stained, even though the bile ducts show a strong staining reaction. The basic protocol settings are similar to the protocol used in Fig. 5-6a, but on a different platform. This shows the importance to carefully calibrate the individual antibody on different platforms and total level of the analytical sensitivity. Same protocol for Fig. 6.b.

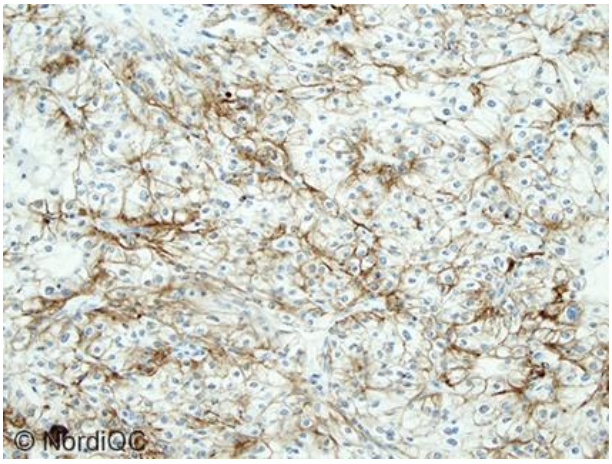



Fig. 6a (x200)
Optimal CK-PAN staining of the CCRCC using same protocol as Fig. 5a. This protocol provide a moderate, distinct cytoplasmic staining reaction with membranous accentuation of virtually all the neoplastic cells.

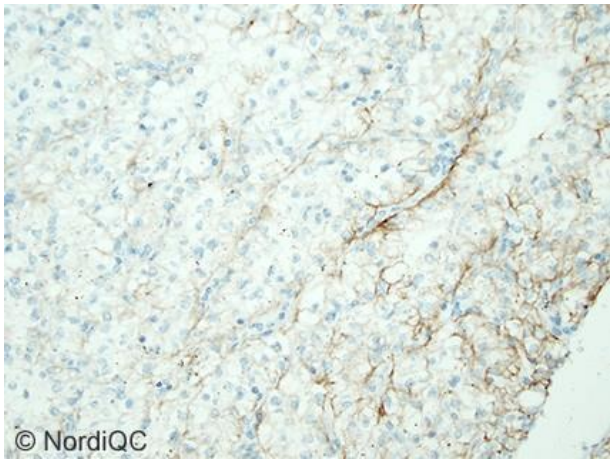



Fig. 6b (x200)
Insufficient CK-PAN staining of the CCRCC using the same protocol as Fig 5b. The vast majority of neoplastic cells are virtually all false negative and only dispersed cells at the edge of the tissue core show a weak to moderate membranous staining reaction.

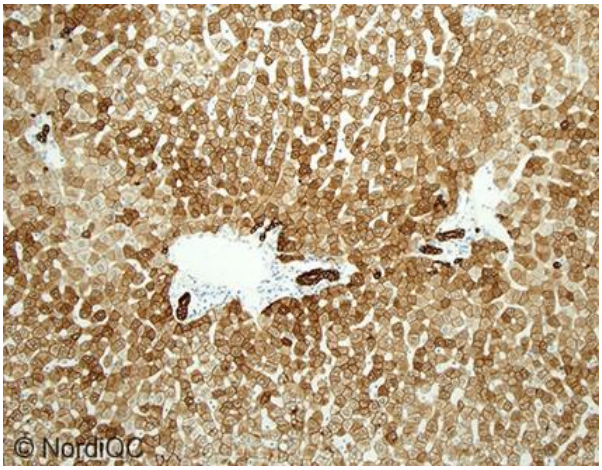



Fig. 7a (x100)
Optimal CK-PAN staining of the liver using the mAb clone BS5 (Nordic Biosite) on the Leica Bond platform. The antibody was diluted 1:200 (30 min.), HIER in BERS2 (Leica) for 20 min., and with a 3-layer polymer detection system (Bond Refine, Leica). Fig.´s 7a and 7b show the two most critical cores with liver and CCRCC, which are stained optimally.
In this assessment mAb clone BS5 was found to be superior to AE1/AE3 on the Leica Bond IHC platform.

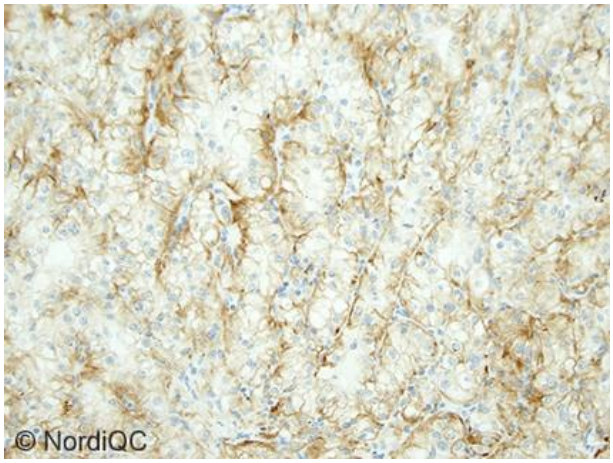



Fig. 7b (x200)
Optimal CK-PAN staining of the CCRCC same protocol as Fig. 7a.
The neoplastic cells show the expected staining pattern.

TJ/LE/SN/RR 31.03.2020

# FLEX Monoclonal Mouse Anti-Human Cytokeratin Clone AE1/AE3 Ready-to-Use (Link)

Technins specifikacijos 29 reikalavimas

**Code IR053**

**Intended use**

For in vitro diagnostic use.

FLEX Monoclonal Mouse Anti-Human Cytokeratin, Clone AE1/AE3, Ready-to-Use, (Link), is intended for use in immunohistochemistry (IHC) with Autostainer Link instruments. This antibody is a useful aid for the classification of tumors of epithelial origin (1-3). Differential classification is aided by the results from a panel of antibodies. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

**Summary and explanation**

Cytokeratins are a family of water-soluble proteins with molecular weights between 40-70 kDa that form the cytoskeleton of epithelial cells. At least 19 different cytokeratins have been identified and can be divided into two subfamilies. Subfamily A comprises relatively acidic cytokeratins (with a pH under 5.5) whereas members of subfamily B have a relatively basic pH of 6 or over.

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure, Materials Required, Not Supplied, Storage, Specimen Preparation, Staining Procedure, Quality Control, Troubleshooting, Interpretation of Staining, General Limitations.

**Reagent provided**

Ready-to-use monoclonal mouse antibody provided in liquid form in a buffer containing stabilizing protein and 0.015 mol/L $NaN_3$.

Clone: AE1/AE3. Isotype: IgG1, kappa.

**Immunogen**

Human epidermal callus (1).

**Specificity**

AE1/AE3 is a cocktail of two monoclonal antibodies that were obtained by immunizing mice with human callus keratins (2). AE1/AE3 has been shown to identify the majority of human cytokeratins and thus may be used as a tool for the positive IHC identification of cells of simple and stratified epithelial origin (1,2,4). Antibody AE1 immunoreacts with an antigenic determinant present on most of the subfamily A cytokeratins, including cytokeratins with Moll's designation (4) 10, 13, 14, 15 16 and 19 (MWs of 56.5, 54', 50, 50', 48 and 40 kDa, respectively) but not on Nos. 12, 17 and 18 (55, 47 and 45 kDa) (4). Antibody AE3 reacts with an antigenic determinant shared by the subfamily B cytokeratins including Nos. 1 and 2, 3, 4, 5, 6, 7 and 8 (MWs of 65, 67, 64, 59, 58, 56, 54 and 52 kDa, respectively) (5).

**Precautions**

1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused solution should be disposed of according to local, State and Federal regulations.

**Storage**

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

**Specimen preparation**

Paraffin sections: The antibody can be used for labeling formalin-fixed, paraffin-embedded tissue sections. Tissue specimens should be cut into sections of approximately 4 µm.

Pre-treatment: Pre-treatment with heat-induced epitope retrieval (HIER) is required. Optimal results are obtained by pretreating tissues using EnVision FLEX Target Retrieval Solution, High pH (50x) (Code K8004).

Deparaffinized sections: Pre-treatment of deparaffinized formalin-fixed, paraffin-embedded tissue sections is recommended using Dako PT Link. For details, please refer to the PT Link User Guide. The following parameters should be used for PT Link: Pre-heat temperature: 65 °C; epitope retrieval temperature and time: 97 °C for 20 (±1) minutes; cool down to 65 °C. Rinse sections with diluted room temperature EnVision FLEX Wash Buffer (20x) (Code K8007).

Paraffin-embedded sections: As alternative specimen preparation, both deparaffinization and epitope retrieval can be performed in the PT Link using a modified procedure. See the PT Link User Guide for instructions. After the staining procedure has been completed, the sections must be dehydrated, cleared and mounted using permanent mounting medium.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of Dako Silanized Slides (Code S3003) is recommended. After staining the sections must be dehydrated, cleared and mounted using a permanent mounting method.

**Staining procedure**

The recommended visualization system is EnVision FLEX, High pH (Link) (Code K8000). The staining steps and incubation times are pre-programmed into the Autostainer Link software. Please refer to the proper Autostainer Link User Guide for detailed instructions on loading slides and reagents. If the protocols are not available on the used Autostainer platform, please contact Dako Technical Support. All incubation steps should be performed at room temperature.

Optimal conditions may vary depending on specimen and preparation methods, and should be determined by each individual laboratory.

Counterstaining in hematoxylin is recommended using EnVision FLEX Hematoxylin (Link) (Code K8008).

Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include squamous mucosa and the cells/structures should display reaction patterns as described for this tissue in “Performance characteristics”. The recommended negative control reagent is FLEX Negative Control, Mouse (Link) (Code IR750).

**Staining interpretation**

The cellular staining pattern is cytoplasmic.

**Performance characteristics**

Normal tissues: Testing of 30 different normal tissues demonstrated staining in the cytoplasm of squamous and columnar epithelium of the cervix, colon, esophagus, skin, small intestine, stomach and tonsil. Other tissues that stained included glandular tissue (mammary, parathyroid, prostate sweat and thyroid), astrocyte, white matter of the cerebellum, glial filaments of the cerebrum, distal tubule and Bowman’s capsule of the kidney, bile duct, pneumocytes, bronchi, mesothelium, interlobular duct of the pancreas, anterior pituitary cell, interlobular duct and acinar cells of the salivary gland, reticular cells and Hassall’s bodies of the thymus, and endometrium and smooth muscle of the uterus (6). No staining was noted for adrenal, bone marrow, heart, pericardium, peripheral nerve, skeletal muscle, spleen and testis.

AE1/AE3 reacts with keratinized (56.5/65-67) and corneal (55/64) epidermis, stratified squamous epithelia of internal organs (51/59), stratified epithelia (50/58), hyperproliferative keratinocytes (48/56) and simple epithelia (45/52 and 46/54). The 40 kDa keratin is present in most epithelia except adult epidermis (3,4).

Abnormal tissues: In pathological tissues, Listrom and Dalton (7) tested clones AE1/AE3 on over 60 poorly differentiated epithelial neoplasms, lymphomas, melanomas and sarcomas. Except for staining of only 2/6 cases of small cell carcinoma and 3/5 transitional cell carcinoma, the study found all of 34 epithelial neoplasms to stainwith AE1/AE3. When reactive, AE1/AE3 stained transitional cell carcinomas only weakly and staining of the tumor cells was either diffusely cytoplasmic or perinuclear. Montag et al. (8) found AE1/AE3 to be a sensitive reagent for the classification of diffuse malignant mesothelioma of the sarcomatoid (spindle-cell) type (labeled in 30/30 cases) whereas other types of spindle cell neoplasms were unreactive (0/49). When compared with anti-EMA in a study of 87 neoplasms, including 48 adenocarcinomas of various types, Pinkus et al. (6) found AE1/AE3 to stain 33% of the cases more reliably than the anti-EMA.

Although 3/3 cases of chondroid chordoma and 1/8 cases of lymphoma were reactive with anti-AE1/AE3, no staining was observed among 25 non-epithelial neoplasms including 4 cases each of melanoma and glioblastoma (7).

**References**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4. Sun T-T, Eichner R, Schermer A, Cooper D, Nelson WG, Weiss RA. Classification, expression and possible mechanisms of evolution of mammalian epithelial keratins: A unifying model. In: Levine A, Topp W, Vande Woude G, Watson JD (eds.). Cancer cells 1 the transformed phenotype. New York: Cold Spring Harbor Laboratory 1984
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6. Pinkus GS, Etheridge CL, O'Connor EM. Are keratin proteins a better tumor marker than epithelial membrane antigen? Amer J Clin Pathol 1986; 85:269
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8. Montag AG, Pinkus GS, Corson JM. Keratin protein immunoreactivity of sarcomatoid and mixed types of diffuse malignant mesothelioma: An immunoperoxidase study of 30 cases. Hum Pathol 1988; 19:336
9. Nelson WG, Battifora H, Santana H and Sun T-T. Specific keratins as molecular markers for neoplasms with a stratified epithelial origin. Canc Res 1984; 44:1600


**Explanation of symbols**

| | | | | | |
|---|---|---|---|---|---|
| REF | Catalogue number | | Temperature limitation | IVD | In vitro diagnostic medical device |
| | Manufacturer | LOT | Batch code | Σ | Contains sufficient for <n> tests |
| | Use by | | Consult instructions for use | EC REP | Authorized representative in the European Community |


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TX02438/01

Revision 2020.06

# Monoclonal Mouse Anti-Human Cytokeratin Clones AE1/AE3

Technins specifikacijos 20 reikalavimas

**Code M3515**

**Intended use**
For in vitro diagnostic use.

Monoclonal Mouse Anti-Human Cytokeratin, Clones AE1/AE3 is intended for use in immunohistochemistry (IHC). The antibody identifies two epitopes present on a majority of epithelial cytokeratins in formalin-fixed, paraffin-embedded tissue. Results aid in the classification of normal and neoplastic tissue as epithelial in origin[1-3]. Differential classification of tumors is aided by the results from a panel of antibodies. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

**Summary and explanation**
Cytokeratins are a family of water-soluble proteins with molecular weights between 40-70 kDa that form the cytoskeleton of epithelial cells. At least 19 different cytokeratins have been identified and can be divided into two subfamilies. Subfamily A comprises relatively acidic cytokeratins (with a pI under 5.5) whereas members of subfamily B have a relatively basic pI of 6 or over.

Refer to *Dako General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: Principle of Procedure, Materials Required, Not Supplied, Storage, Specimen Preparation, Staining Procedure, Quality Control, Troubleshooting, Interpretation of Staining, General Limitations.

**Reagent provided**
Monoclonal mouse antibody provided in liquid form as tissue culture supernatant in 0.05 mol/L Tris-HCl, pH 7.2 and 0.015 mol/L sodium azide. This product contains stabilizing protein.

Clones: AE1/AE3[1,2] Isotype: IgG1, kappa
Mouse IgG concentration: See label on vial.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

**Immunogen**
Human epidermal callus[1]

**Specificity**
AE1/AE3 is a cocktail of two monoclonal antibodies that were obtained by immunizing mice with human callus keratins.[2] AE1/AE3 has been shown to identify the majority of human cytokeratins and thus may be used as a tool for the positive IHC identification of cells of simple and stratified epithelial origin.[1,2,4] Antibody AE1 immunoreacts with an antigenic determinant present on most of the subfamily A cytokeratins, including cytokeratins with Moll's designation[4] 10, 13, 14, 15 16 and 19 (MWs of 56.5, 54', 50, 50', 48 and 40 kDa, respectively) but not on Nos. 12, 17 and 18 (55, 47 and 45 kDa).[4] Antibody AE3 reacts with an antigenic determinant shared by the subfamily B cytokeratins including Nos. 1 and 2, 3, 4, 5, 6, 7 and 8 (MWs of 65, 67, 64, 59, 58, 56, 54 and 52 kDa, respectively).[5]

**Materials required, but not supplied**
Refer to *Dako General Instructions for Immunohistochemical Staining* and/or the detection system instructions.

**Precautions**
1. For in vitro diagnostic use.
2. For professional users.
3. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, $NaN_3$ may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
4. As with any product derived from biological sources, proper handling procedures should be used.
5. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
6. Unused reagents should be disposed of according to local, State, and Federal regulations.

**Storage**
Store at 2–8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

**Specimen preparation**
Paraffin sections: The antibody can be used for labelling formalin-fixed, paraffin-embedded tissue sections fixed in formalin. Tissue specimens should be cut into sections of approximately 4 µm.

Pretreatment: Pre-treatment of deparaffinized tissues with proteolytic enzymes or heat-induced epitope retrieval is recommended. For heat-induced epitope retrieval optimal results are obtained with EnVision FLEX Target Retrieval Solution, High pH (50x) (Code K8004). Epitope retrieval can be performed in Dako PT Link. For details, please refer to PT Link User Guide.

As an alternative to heat induced epitope retrieval, enzyme pretreatment can be used. The following enzymes can be used for pretreatment of formalin-fixed, paraffin-embedded tissues: Proteinase K, RTU (Code S3020), Pepsin (Code S3002), or Proteolytic Enzyme, RTU (Code S3007). Rinse thoroughly with distilled water and continue with the staining procedure of the detection system instructions.

The tissue sections should not dry out during the treatment or during the following immunohistochemical staining procedure. For greater adherence of tissue sections to glass slides, the use of FLEX IHC Microscope Slides (Code K8020) is recommended. After staining, the sections must be dehydrated, cleared and mounted using a permanent mounting method.

**Staining procedure**
These are guidelines only. Optimal conditions may vary depending on specimen type and preparation method, and should be validated individually by each laboratory. The performance of this antibody should be established by the user when utilized with other manual staining systems or automated platforms.

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Dilution: M3515 may be used at a dilution of 1:50 when performing IHC using the EnVision FLEX, EnVision or LSAB2 detection systems. Follow the procedure enclosed with the selected visualization system(s).

Quality control: Positive and negative control tissues as well as negative control reagent should be run simultaneously using the same protocol as the patient specimens.

**Product specific limitations**
1. Extrafollicular reticulum cells of lymph nodes, tonsil and spleen have been shown to react with antibodies to cytokeratin 8.[6]
2. The presence of cytokeratin 19 and possibly 8 has been confirmed in smooth muscle cells of the uterus.[7]
3. Rare melanomas[8] and leiomyosarcomas[7] may stain positive. This finding is usually more pronounced on frozen tissue rather than formalin-fixed tissue.[9]
4. False-positive staining of glial cells in tumors has been reported on formalin-fixed, paraffin-embedded tissue when proteolytic pretreatment was employed. It was shown by immunocytochemical and biochemical methods that these cells and tumors do not express cytokeratins.[10]
5. Pinkus et al.[11] stressed the importance of proteolytic digestion of formalin-fixed tissues to be stained with AE1/AE3. Photos of stained tissues depicted include the results when digestion with trypsin II was omitted, other trypsin enzymes were used and/or when suboptimal digestion techniques were applied. In the latter cases, only 2/12 epithelial neoplasms of various types exhibited optimal staining for cytokeratin. By reviewing conflicting cytokeratin immunoreactivities in earlier publications and comparing the same with their own, Pinkus et al.[11] considered many previously false-negative cases attributable to one or several of these short-comings.
6. From a comparison of AE1/AE3 with an anti-epithelial membrane antigen (EMA) antibody in an IHC study of 87 neoplasms, including 48 adenocarcinomas of various types, Pinkus et al.[12] concluded that the cytokeratin proteins in proteolytically treated formalin-fixed tissues and as stained by AE1/AE3 were more reliable markers in 33% of the cases of epithelial derived neoplasms than anti-EMA. However, because EMA was labeled and AE1/AE3 unlabeled in 9% of the cases, it was recommended that AE1/AE3 and anti-EMA be used as complementary reagents.
7. Although no false-positive staining was reported by Listrom and Dalton,[13] faint cytoplasmic staining was observed in 2/2 plasmacytomas, 2/4 melanomas and 2/7 lymphomas and considered to be the result of nonspecific background staining.

**Staining interpretation**
The cellular staining pattern for AE1/AE3 is cytoplasmic.

**Performance characteristics**
Normal tissues: Testing of 30 different normal tissues demonstrated staining in the cytoplasm of squamous and columnar epithelium of the cervix, colon, esophagus, skin, small intestine, stomach and tonsil. Other tissues that stained included glandular tissue (mammary, parathyroid, prostate sweat and thyroid), astrocyte, white matter of the cerebellum, glial filaments of the cerebrum, distal tubule and Bowman's capsule of the kidney, bile duct, pneumocytes, bronchi, mesothelium, interlobular duct of the pancreas, anterior pituitary cell, interlobular duct and acinar cells of the salivary gland, reticular cells and Hassall's bodies of the thymus, and endometrium and smooth muscle of the uterus.[14] No staining was noted for adrenal, bone marrow, heart, pericardium, peripheral nerve, skeletal muscle, spleen and testis.

AE1/AE3 reacts with keratinized (56.5/65-67) and corneal (55/64) epidermis, stratified squamous epithelia of internal organs (51/59), stratified epithelia (50/58), hyperproliferative keratinocytes (48/56) and simple epithelia (45/52 and 46/54). The 40 kDa keratin is present in most epithelia except adult epidermis.[3,4]

Abnormal tissues: In pathological tissues, Listrom and Dalton[13] tested clones AE1/AE3 on over 60 poorly differentiated epithelial neoplasms, lymphomas, melanomas and sarcomas. Except for staining of only 2/6 cases of small cell carcinoma and 3/5 transitional cell carcinoma, the study found all of 34 epithelial neoplasms to stain. When labeled, AE1/AE3 stained transitional cell carcinomas only weakly and staining of the tumor cells was either diffusely cytoplasmic or perinuclear. Montag et al.[15] found AE1/AE3 to be a sensitive reagent for the classification of diffuse malignant mesothelioma of the sarcomatoid (spindle-cell) type (positive in 30/30 cases). When compared with anti-EMA in a study of 87 neoplasms, including 48 adenocarcinomas of various types, Pinkus et al.[12] found AE1/AE3 to stain 33% of the cases more reliably than the anti-EMA.

Although 3/3 cases of chondroid chordoma and 1/8 cases of lymphoma were reactive with anti-AE1/AE3, no staining was observed among 25 non-epithelial neoplasms including 4 cases each of melanoma and glioblastoma.[13]

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**Explanation of symbols**

| | | | | | |
|---|---|---|---|---|---|
| REF | Catalogue number | | Temperature limitation | IVD | In vitro diagnostic medical device |
| | Manufacturer | LOT | Batch code | Σ | Contains sufficient for <n> tests |
| | Use by | | Consult instructions for use | EC REP | Authorized representative in the European Community |

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CE

# Monoclonal Mouse Anti-Human PD-L1
Clone 22C3

**Code M3653**

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**Intended use**

For in vitro diagnostic use.
Monoclonal Mouse Anti-Human PD-L1, Clone 22C3, is intended for use in immunohistochemistry. This antibody labels PD-L1 in normal and neoplastic tissue. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a certified pathologist. This antibody is intended to be used after the primary diagnosis of tumor has been made by conventional histopathology using nonimmunologic histochemical stains.

**Synonyms for antigen**

Programmed Death-Ligand 1 *(1).*

**Summary and explanation**

Binding of the PD-1 ligands, PD-L1 and PD-L2, to the PD-1 receptor found on T cells, inhibits T cell proliferation and cytokine production (2). Up-regulation of PD-1 ligands occurs in some tumors and signaling through this pathway can contribute to inhibition of active T-cell immune surveillance of tumors (3). Blockade of PD-1 interaction with PD-L1 and PD-L2 releases PD-1 pathway-mediated inhibition of the immune response, including the anti-tumor immune response (4).

Refer to our *General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures.

**Reagent provided**

Monoclonal mouse antibody provided in liquid form in 0.05 mol/L Tris-HCl, 0.015 mol/L sodium azide, 1% bovine serum albumin, pH 7.2.

Clone: 22C3 (5). Isotype: IgG1.
Mouse IgG concentration mg/L: See label on vial.

The protein concentration between lots may vary without influencing the optimal dilution. The titer of each individual lot is compared and adjusted to a reference lot to ensure a consistent immunohistochemical staining performance from lot-to-lot.

**Immunogen**

Human extracellular domain of PD-L1 (Phe19-Thr239) fused to a human IgG1 fragment (R&D Systems Catalogue No. 156-B7-100)

**Specificity**

In Western blotting of recombinant human PD-L1 protein, Monoclonal Mouse Anti-Human PD-L1, Clone 22C3 labels a band corresponding to ~40 kDa.

**Precautions**

1. For professional users.
2. This product contains sodium azide ($NaN_3$), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused solution should be disposed of according to local, State and Federal regulations.

**Storage**

Store at 2-8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Agilent Pathology Support.

**Specimen preparation**

Paraffin sections: The antibody can be used for labeling paraffin-embedded tissue sections fixed in formalin. Tissue specimens should be cut into sections of approximately 4 µm.

Pre-treatment: Please refer to EnVision™ FLEX Target Retrieval Solution, Low pH (50x) (Code K8005) for instructions on how to perform pretreatment of slides.

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**Staining procedure**

These are guidelines only. Optimal conditions may vary depending on specimen type and preparation method, and should be validated individually by each laboratory. The performance of this antibody should be established by the user when utilized with other manual staining systems or automated platforms.

Dilution: The recommended dilution of Monoclonal Mouse Anti-Human PD-L1, Clone 22C3, Code M3653, is 1:50. Dilute the antibody in Dako Antibody Diluent with Background-Reducing Components (Code S3022). Incubate pretreated tissue sections for 30 minutes at room temperature.

Negative control: The recommended negative control reagent is Dako Negative Control, Mouse IgG1 (Code X0931) diluted to the same IgG1 concentration as the primary antibody. Unless the stability of the diluted antibody and negative control has been established in the actual staining procedure, dilute these reagents immediately prior to use. Positive and negative controls should be run simultaneously with patient specimens.

Visualization: The recommended EnVision™ FLEX HRP visualization system is using 30-minute incubation at room temperature. The incubation time for EnVision™ FLEX+ Mouse (LINKER) (Code K8021/K8022) is 30 minutes at room temperature. Follow the procedure enclosed with the selected visualization system(s).

Note: Use EnVision™ FLEX Target Retrieval Solution, **Low pH** (50x) (Code K8005) for HIER.

The incubation time for DAB enhancer (S196131-2) is 5 minutes at room temperature.

Counterstaining: The recommended counterstain is EnVision™ FLEX Hematoxylin (Code K8008/K8018). For optimal results, non-aqueous, permanent mounting medium is recommended.

Controls: Positive and negative control tissues should be run simultaneously using the same protocol as the patient specimens. The positive control tissue should include tonsil and the cells/structures should display reaction patterns as described for this tissue in the "Performance characteristics" section.

**Staining interpretation**

The cellular staining pattern is membranous.

**Performance characteristics**

Normal tissues:

Plasma membrane staining was observed on immune cells and cells of epithelial origin. Cytoplasmic staining was noted in some cell types but was not recorded as positive staining. Table 1 summarizes PD-L1 immunoreactivity on the recommended panel of normal tissues. All tissues were formalin-fixed and paraffin-embedded and stained with Monoclonal Mouse Anti-PD-L1, Clone 22C3 according to the instructions in this package insert. There were no unexpected results observed in cell types or tissue types tested. The observed staining was consistent with the reported literature for PD-L1 IHC expression in normal tissues (1, 2).

**Table 1: Summary of Monoclonal Mouse Anti-PD-L1, Clone 22C3 normal tissue reactivity**

| Tissue Type (# tested) | Positive Tissue Elements | Tissue Type (# tested) | Positive Tissue Elements |
|---|---|---|---|
| Adrenal (3) | 0/3 | Parathyroid (3) | 1/3 Glandular epithelium |
| Bone marrow (3) | 3/3 Megakaryocyte | | |
| Breast (3) | 0/3 | Pancreas (3) | 0/3 |
| Cerebellum (3) | 0/3 | Pituitary (3) | 1/3 Anterior hypophysis |
| Cerebrum (3) | 0/3 | | 1/3 Posterior hypophysis |
| Cervix (3) | 1/3 epithelial cells | Prostate (2) | 2/2 epithelium |
| Colon (3) | 1/3 Lymphocytes | Salivary Gland (3) | 0/3 |
| | 1/3 Macrophages | | |
| Esophagus (3) | 0/3 | Skin (3) | 0/3 |
| Kidney (3) | 1/3 Tubular epithelium | Small Intestine (3) | 0/3 |
| Liver (3) | 1/3 Macrophages | Spleen (3) | 2/3 Macrophages |
| | 1/3 Hepatocytes | | |
| Lung (3) | 3/3 Macrophages | Stomach (3) | 2/3 Lymphocytes |
| | | | 1/3 gastric glands |
| Mesothelial cells (2) | 0/2 | Testis (3) | 0/3 |
| Muscle, cardiac (3) | 0/3 | Thymus (3) | 3/3 Medulla |
| Muscle, skeletal (3) | 0/3 | Thyroid (3) | 0/3 |
| Nerve, peripheral (3) | 0/3 | Tonsil (3) | 3/3 Crypt epithelium |
| | | | 2/3 macrophages |
| Ovary (3) | 0/3 | Uterus (3) | 0/3 |

Abnormal tissues:

Plasma membrane staining was observed on immune cells and cells of epithelial origin. Cytoplasmic staining was noted in some cell types but was not recorded as positive staining. Table 2 summarizes PD-L1 immunoreactivity on a panel of neoplastic tissues. All tissues were formalin-fixed and paraffin-embedded and stained with Monoclonal Mouse Anti-PD-L1, Clone 22C3 according to the instructions in this package insert. There were no unexpected results observed in the tumor specimens tested. The

observed staining was consistent with the reported literature for PD-L1 IHC expression in neoplastic tissues (1-4).

**Table 2: Summary of Monoclonal Mouse Anti-PD-L1, Clone 22C3 neoplastic tissue reactivity**

| Tumor Type | Location | PD-L1 positive/ N=159 |
|---|---|---|
| Adenocarcinoma | Appendix | 0/1 |
| | Breast, DCIS | 0/2 |
| | Breast, invasive ductal | 0/7 |
| | Breast, invasive ductal metastatic to lymph node | 0/1 |
| | Cervix, endocervical type | 0/1 |
| | Colon | 0/5 |
| | Colon, metastatic to liver | 0/1 |
| | Colon, mucinous | 0/1 |
| | Esophagus | 0/1 |
| | Gallbladder | 1/5 |
| | GI, metastatic to lung | 0/1 |
| | Head & neck, hard palate | 0/1 |
| | Lung | 1/4 |
| | Ovary | 0/1 |
| | Ovary, endometrioid | 0/1 |
| | Ovary, mucinous | 0/1 |
| | Ovary, serous | 0/1 |
| | Pancreas | 0/2 |
| | Pancreas, ductal | 0/3 |
| | Prostate | 0/5 |
| | Rectum | 0/4 |
| | Salivary/parotid gland | 0/2 |
| | Small intestine | 0/2 |
| | Stomach | 0/6 |
| | Stomach, mucinous | 0/1 |
| | Thyroid, follicular | 0/1 |
| | Thyroid, follicular-papillary | 0/1 |
| | Thyroid, papillary | 0/3 |
| | Uterus, clear cell | 0/1 |
| | Uterus, endometrium | 0/3 |
| Adrenocortical carcinoma | Adrenal | 0/1 |
| Astrocytoma | Cerebrum | 0/3 |
| Basal cell carcinoma | Skin | 0/1 |
| Carcinoma | Nasopharyngeal, NPC | 0/1 |
| Chondrosarcoma | Bone | 0/1 |
| Chordoma | Pelvic cavity | 0/1 |
| Embryonal carcinoma | Testis | 0/1 |
| Ependymoma | Brain | 0/1 |
| Glioblastoma | Brain | 0/1 |
| Hepatoblastoma | Liver | 0/1 |
| Hepatocellular carcinoma | Liver | 0/5 |
| Islet Cell tumor | Pancreas | 0/1 |
| Interstitialoma | Colon | 0/1 |
| | Rectum | 0/1 |
| | Small intestine | 0/1 |
| Leiomyosarcoma | Soft tissue, chest wall | 0/1 |
| | Bladder | 0/1 |

| | | |
|---|---|---|
| Lymphoma | | |
| Anaplastic Large Cell | Lymph node | 0/1 |
| Diffuse B-cell | Lymph node | 0/4 |
| Hodgkin | Lymph node | 2/2 |
| Non-Hodgkin | Lymph node | 1/1 |
| Medulloblastoma | Brain | 0/1 |
| Medullary carcinoma | Thyroid | 0/1 |
| Melanoma | Rectum | 0/1 |
| | Nasal cavity | 0/1 |
| Meningioma | Brain | 0/2 |
| Mesothelioma | Peritoneum | 0/1 |
| Neuroblastoma | Retroperitoneum | 0/1 |
| Neurofibroma | Soft tissue, lower back | 0/1 |
| Osteosarcoma | Bone | 0/2 |
| Pheochromocytoma | Adrenal | 0/1 |
| Primitive Neuroectodermal Tumor (PNET) | Retroperitoneum | 0/1 |
| Renal Cell carcinoma | | |
| Papillary | Kidney | 0/1 |
| Clear Cell | Kidney | 0/6 |
| Rhabdomyosarcoma | Soft tissue, embryonal | 0/1 |
| | Prostate | 0/1 |
| | Retroperitoneum | 0/1 |
| Seminoma | Testis | 0/2 |
| Signet Ring Cell carcinoma | Metastatic colon signet ring cell carcinoma to ovary | 0/1 |
| | Colon | 0/1 |
| Small cell carcinoma | Lung | 0/1 |
| Spermatocytoma | Testis | 0/2 |
| Squamous Cell carcinoma | Metastatic esophageal squamous cell carcinoma to lymph node | 0/1 |
| | Cervix | 2/5 |
| | Esophagus | 0/7 |
| | Head & neck | 0/2 |
| | Lung | 1/2 |
| | Skin | 0/2 |
| | Uterus | 0/1 |
| Synovial Sarcoma | Pelvic cavity | 0/1 |
| Thymoma | Mediastinum | 1/1 |
| Transitional Cell carcinoma | Bladder | 0/6 |
| | Kidney | 0/1 |

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**Explanation of symbols**

| | | | | | |
|---|---|---|---|---|---|
| REF | Catalogue number |  | Temperature limitation | IVD | In vitro diagnostic medical device |
|  | Manufacturer | LOT | Batch code |  | Contains sufficient for <n> tests |
|  | Use by |  | Consult instructions for use | EC REP | Authorized representative in the European Community |


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