

CONFIRM anti-Cytokeratin 20 (SP33) Rabbit Monoclonal Primary Antibody

REF 790-4431

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IVD  50

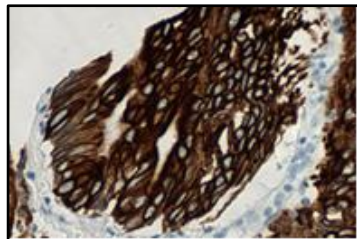


Figure 1. CONFIRM anti-Cytokeratin 20 (SP33) Rabbit Monoclonal Primary Antibody cytoplasmic staining of colon adenocarcinoma.

cytokeratin 20 expressing cells and neoplastic cells of colonic epithelial lineage. The antibody is intended for qualitative staining in sections of formalin fixed, paraffin embedded tissue.

The clinical interpretation of any staining, or the absence of staining, must be complemented by histological studies and evaluation of proper controls. Evaluation must be made by a qualified pathologist within the context of the patient's clinical history and other diagnostic tests.

SUMMARY AND EXPLANATION

CONFIRM anti-Cytokeratin 20 (SP33) Rabbit Monoclonal Primary Antibody stains intestinal and gastric epithelium, urothelium and Merkel cells in the epidermis.¹⁻⁴ Tumors derived from cytokeratin 20 expressing epithelia are usually positive;^{3,5} these include most colorectal carcinomas, some gastric adenocarcinomas, as well as adenocarcinomas of the gall bladder, bile duct, and pancreatic ducts. Mucinous ovarian carcinomas, transitional cell carcinomas and Merkel cell carcinomas are also usually positive.^{4,6} This antibody recognizes intermediate filaments in the cytoplasm of positive cells.¹

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-Cytokeratin 20 antibody is a rabbit monoclonal antibody raised against the carboxyl terminus of the human Cytokeratin 20 protein. This antibody can be visualized using *ultraView* Universal DAB Detection Kit (Cat. No. 760-500; Order No. 05269806001). Refer to the *ultraView* Universal DAB Detection Kit package inserts for further information.

REAGENT PROVIDED

CONFIRM anti-Cytokeratin 20 (SP33) Rabbit Monoclonal Primary Antibody contains sufficient reagent for staining 50 slides.

One 5 mL dispenser of CONFIRM anti-Cytokeratin 20 antibody contains approximately 9.5 µg of a rabbit monoclonal (SP33) antibody.

The antibody is diluted in 0.05 M Tris-HCL with 0.3% carrier protein and 0.10% ProClin 300, a preservative.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 1.9 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-Cytokeratin 20 antibody is a recombinant rabbit monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the package insert may be available in all geographies. Consult your local support representative.

STORAGE

Upon receipt and when not in use, store at 2 to 8°C. Do not freeze

To ensure proper reagent delivery and stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with Ventana detection kits and VENTANA BenchMark XT and BenchMark ULTRA automated slide stainers. The recommended tissue fixative is 10% neutral buffered formalin.⁷ Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic (IVD) use.
2. For professional use only.
3. ProClin 300 solution is used as a preservative in this reagent. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
4. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
5. This product contains 1% or less bovine serum which is used in the manufacture of the antibody.
6. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
7. Avoid microbial contamination of reagents as it may cause incorrect results.
8. Consult local and/or state authorities with regard to recommended method of disposal.
9. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Hazard Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on VENTANA BenchMark XT and BenchMark ULTRA automated slide stainers in combination with VENTANA detection kits and accessories. Refer to Table 1 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instrument's Operator's Manual. Refer to the appropriate VENTANA detection package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocols for CONFIRM anti-Cytokeratin 20 antibody with *ultraView* Universal DAB Detection Kit on a BenchMark XT instrument and BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument 16 minutes, 37°C BenchMark ULTRA instrument 16 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing Reagent, 4 minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances."⁸

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are adenocarcinomas of the colon or tissue containing goblet cells and/or enterocytes.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-Cytokeratin 20 Rabbit Monoclonal Primary Antibody is cytoplasmic.

SPECIFIC LIMITATIONS

CONFIRM anti-Cytokeratin 20 antibody has been optimized on Ventana BenchMark XT and BenchMark ULTRA instruments in combination with *ultraView* Universal DAB Detection Kit (REF 760-500) at a 16 minute primary antibody incubation time; however, the user must validate individual laboratory results obtained with this reagent. Several tissues demonstrated interspersed background staining of macrophages, but the pattern acquired in the macrophages is not similar to the specific staining pattern of cytokeratin 20 in positive tissues.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed in Table 2 and Table 3 and in the Repeatability section.

Specificity

Table 2. Specificity of CONFIRM anti-Cytokeratin 20 antibody was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/2	Esophagus	0/3
Ovary	0/3	Stomach	3/3
Pancreas	0/3	Small intestine	3/3
Parathyroid gland	0/3	Colon ^a	34/34
Hypophysis	0/3	Liver	0/3
Testis	0/3	Salivary gland	0/3
Thyroid	0/3	Kidney	0/3
Breast	0/3	Prostate	0/3

Tissue	# positive / total cases	Tissue	# positive / total cases
Spleen	0/3	Endometrium	0/3
Tonsil	0/3	Cervix	0/3
Thymus	0/3	Skeletal muscle	0/3
Bone marrow	0/3	Skin	1/3
Heart	0/3	Nerve (sparse)	0/3
Mesothelium of lung	0/3	Lung	0/3

a. Tissue category includes non-neoplastic inflamed or diseased cases.

Sensitivity

Table 3. Sensitivity of CONFIRM anti-Cytokeratin 20 antibody was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	0/1
Atypical meningioma	0/1
Malignant ependymoma	0/1
Malignant oligodendroglioma	0/1
Ovarian serous papillary adenocarcinoma	0/1
Ovarian mucinous papillary adenocarcinoma	0/1
Pancreatic islet cell carcinoma	0/1
Pancreatic adenocarcinoma	0/1
Seminoma	0/1
Embryonal carcinoma	0/1
Thyroid medullary carcinoma	0/1
Thyroid papillary carcinoma	0/1
Breast intraductal carcinoma	0/1
Breast invasive ductal carcinoma	0/1
Breast lobular carcinoma in situ ⁹	1/1
Lung squamous cell carcinoma	0/1
Lung adenocarcinoma	0/1
Lung small cell undifferentiated carcinoma	1/1
Esophagus squamous cell carcinoma	0/1
Esophagus adenocarcinoma	0/1
Colon adenocarcinoma	54/65
Colon mucinous adenocarcinoma	13/13
Colon signet ring cell adenocarcinoma	2/2
Colon adenoma	2/2
Colon villotubulous adenoma	3/3
Colon adenomatous polyps	3/3

Pathology	# positive / total cases
Colon hyperplastic polyps	2/2
Colon squamous cell carcinoma	1/5
Stomach mucinous adenocarcinoma	0/1
Small intestine adenocarcinoma	1/1
GIST	0/3
Rectum adenocarcinoma	0/1
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Clear cell carcinoma	0/1
Prostate adenocarcinoma	0/1
Prostate transitional cell carcinoma	1/1
Leiomyoma	0/1
Endometrial adenocarcinoma	0/1
Clear cell carcinoma of endometrium	0/1
Cervical squamous cell carcinoma	0/2
Embryonal rhabdomyosarcoma	0/1
Melanoma	0/1
Basal cell carcinoma	0/1
Skin squamous cell carcinoma	0/1
Neurofibroma	0/1
Neuroblastoma of retroperitoneum	0/1
Malignant epithelial mesothelioma	0/1
Lymphoma	0/4
Bladder transitional cell carcinoma	1/1
Leiomyosarcoma	0/2
Osteosarcoma of right femur	0/1
Spindle cell rhabdomyosarcoma	0/1
Metastatic colon adenocarcinoma	11/14
Metastatic colon mucinous adenocarcinoma	9/13
Metastatic colon signet ring cell carcinoma	2/2

Repeatability

Repeatability studies for CONFIRM anti-Cytokeratin 20 antibody were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-run and Inter-run reproducibility on a BenchMark XT instrument.
- Intra-platform reproducibility on the BenchMark XT instrument and the BenchMark ULTRA instrument.
- Inter-platform reproducibility between the BenchMark XT instrument and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

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CONTACT INFORMATION



Ventana Medical Systems, Inc.
1910 E. Innovation Park Drive
Tucson, Arizona 85755
USA
+1 520 887 2155
+1 800 227 2155 (USA)



www.ventana.com



Roche Diagnostics GmbH
Sandhofer Strasse 116
D-68305 Mannheim
Germany