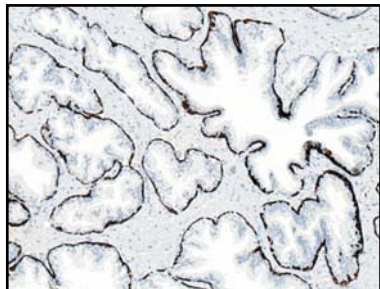


CONFIRM® anti-Keratin (34βE12) Mouse Monoclonal Primary Antibody

Catalog Number 790-4373



INTENDED USE

This antibody is intended for *in vitro* diagnostic use.

Ventana Medical Systems' CONFIRM® anti-Keratin (34βE12) Mouse Monoclonal Primary Antibody recognizes cytokeratins 1, 5, 10, and 14 and is used as an aid in the identification of basal cells in prostate, and myoepithelial cells in breast, via light microscopy in formalin fixed,

paraffin embedded tissue following staining on a Ventana automated slide stainer. The clinical interpretation of any staining, or the absence of staining, must be complemented by morphological 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

Intermediate filaments, including cytokeratins, are distinctive cytoskeletal components which are present in virtually all mammalian cells and are distinguished from other cytoskeletal structures such as microtubules and microfilaments on the basis of filament diameter and protein composition.¹ Filaments containing cytokeratin proteins are characteristic of epithelial cells.² Clone 34βE12 has been characterized as identifying high molecular weight (HMW) cytokeratins found in squamous and ductal epithelium over a wide range of organ tissues.³ Clone 34βE12 reacts with basal cells in normal epithelia of the prostate.⁴ CONFIRM anti-Keratin (34βE12) Mouse Monoclonal Primary Antibody recognizes the 68, 58, 56.5, and 50 kD proteins corresponding to cytokeratins 1, 5, 10, and 14 of the Moll catalog.⁵

REAGENT PROVIDED

CONFIRM anti-Keratin (34βE12) contains sufficient reagent for staining 50 slides.

One 5 mL dispenser of CONFIRM anti-Keratin (34βE12) contains approximately 7 µg of a mouse monoclonal antibody.

The antibody is diluted in 0.05 M Tris-HCl with 2% carrier protein, and 0.10% ProClin™ 300, a preservative containing the active ingredients 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one.

Total protein concentration of the reagent is approximately 10 mg/mL. Specific antibody concentration is approximately 1.4 µg/mL. There is no known irrelevant antibody reactivity observed in this product.

There is a trace (approximately 2%) of fetal bovine serum of U.S. origin from the stock solution.

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 Preparation, (4) Quality Control, (5) Troubleshooting, (6) Interpretation of Staining, and (7) General Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents such as Ventana detection kits (for example, *ultraView*™ Universal DAB detection kit), and ancillary components, including negative and positive tissue control slides, are not provided.

STORAGE

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and stability of the antibody, after every use the cap must be replaced and the dispenser must be immediately placed 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 a Ventana automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.⁶ Heat induced epitope retrieval with an EDTA based basic pH (~8.0) buffer is recommended. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

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

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use.
2. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
3. Avoid microbial contamination of reagents.
4. Consult local or state authorities with regard to recommended method of disposal.
5. The preservative in the reagent is ProClin 300. Symptoms of overexposure to ProClin 300 include skin and eye irritation, and irritation of mucous membranes and upper respiratory tract. The concentration of ProClin 300 in this product is less than or equal to 0.10% and does not meet the OSHA criteria for a hazardous substance. Systemic allergic reactions are possible in sensitive individuals.

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for a BenchMark® XT instrument with *ultraView* Universal DAB detection kit (Cat. No. 760-500) is listed below in Table 1. 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 Protocol for CONFIRM anti-Keratin (34βE12) with *ultraView* Universal DAB Detection Kit on BenchMark XT Instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Standard Cell Conditioning 1
Enzyme (Protease)	None Required
Antibody (Primary)	Approximately 16 Minutes, 37 °C
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bleuing Reagent, 4 Minutes

Due to variation in tissue fixation and processing, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens and detection used. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".⁷

POSITIVE TISSUE CONTROL

Examples of positive control tissues for CONFIRM anti-Keratin (34βE12) are benign prostate (as depicted in the above image). Basal cells in benign and normal prostate glands should stain positively.

STAINING INTERPRETATION

The cellular staining pattern for CONFIRM anti-Keratin (34βE12) is cytoplasmic.

SPECIFIC LIMITATIONS

This antibody has been optimized for a 16 minute incubation time on BenchMark XT automated slide stainers in combination with *ultraView* Universal DAB detection kit (Cat.

No. 760-500), however the user must validate results obtained with this reagent. Clone 34βE12 was found to occasionally exhibit cross-reactivity with simple epithelia and tumors not expected to contain the target molecular weight cytokeratins 1,5,10 and 14. Examples of cross-reactivity seen with this clone in normal tissue are the bile ducts of liver, pancreatic ducts and ovary follicles. In tumors, lung, ovarian and pancreatic tumors were sometimes reactive. As these normal structures and tumors are thought to have cytokeratins 7, 8, 18 and 19, the observed cross-reactivity with clone 34βE12 is presumed to be between cytokeratins 14 and 19, which share several epitopes.

PERFORMANCE CHARACTERISTICS

- Immunoreactivity of CONFIRM anti-Keratin (34βE12) was determined by testing formalin fixed, paraffin embedded normal and neoplastic tissues. For normal tissues, results are as follows: adrenal gland (0/3), bone marrow (0/3), brain cerebrum (0/3), brain cerebellum (0/3), breast (3/3), cervix (3/3), colon (0/3), esophagus (2/3), heart (0/3), hypophysis (0/3), intestine (0/3), kidney (0/3), liver (1/3), lung (0/3), mesothelium (0/3), nerve (0/3), ovary (0/3), pancreas (1/3), parathyroid (0/3), prostate (3/3), salivary gland (3/3), skin (3/3), spleen (0/3), stomach (0/3), striated muscle (0/3), testis (0/3), thymus (3/3), thyroid (0/3), tonsil (3/3), and uterus (2/3). For neoplastic tissues, results are as follows: atypical meningioma (0/1), glioblastoma (0/1), ependymoma (0/1), oligodendroglioma (0/1), ovarian serous papillary adenocarcinoma (0/1), ovarian mucous papillary adenocarcinoma (1/1), islet cell carcinoma (0/1), pancreatic adenocarcinoma (0/1), testicular seminoma and embryonal carcinoma (0/2), medullary thyroid carcinoma (0/1), papillary thyroid carcinoma (1/1), intraductal, lobular, and infiltrating breast carcinoma (2/3), diffuse B-cell lymphoma in spleen (0/1), small cell lung carcinoma (0/1), squamous cell lung carcinoma (1/1), lung adenocarcinoma (0/1), esophageal squamous cell and adenocarcinoma (2/2), adenocarcinoma in stomach (0/1), intestinal adenocarcinoma and mesenchymoma (1/2), colorectal adenocarcinoma and mesenchymoma (1/4), hepatocellular carcinoma (0/1), hepatoblastoma (0/1), clear cell carcinoma (0/1), adenocarcinoma in prostate (0/1), transitional cell carcinoma in prostate and bladder (2/2), uterine leiomyoma (0/1), endometrial carcinoma (1/1), uterine clear cell and squamous carcinomas (3/3), embryonal rhabdomyosarcoma (0/1), rectal melanoma (0/1), basal cell carcinoma in skin (1/1), squamous cell carcinoma in skin (0/1), neurofibroma and neuroblastoma (0/2), mesothelioma (0/1), Hodgkin's lymphoma (0/1), diffuse type lymphoma (0/3), transitional cell carcinoma and leiomyosarcoma in smooth muscle (2/3), osteosarcoma (0/1), and spindle cell rhabdomyosarcoma (0/1).
- Immunoreactivity of CONFIRM anti-Keratin (34βE12) was also evaluated by testing a variety of formalin fixed, paraffin embedded neoplastic prostate and breast tissues. For prostate, 170 samples were stained and evaluate for positivity. When present, the basal cells of normal and benign ducts stained strongly while diminishing signal or negative results were observed in samples with invasive or advanced prostatic carcinoma. For breast, 171 samples were stained and evaluated for positivity. When present, the myoepithelial cells stain positively.
- Inter-run reproducibility was determined by staining 5 replicate slides containing the same 3 tissues from duplicate sample types across the dynamic range over 5 days on a BenchMark XT instrument. 150 of 150 tissues tested scored equivalently.
- Intra-run reproducibility was determined by staining 14 replicate slides containing the same 3 tissues from duplicate samples types across the dynamic range on a BenchMark XT instrument. 84 of 84 tissues tested scored equivalently.
- Intra-platform reproducibility was determined by staining 5 replicate slides containing the same 3 tissues from duplicate sample types across the dynamic range over 3 BenchMark XT instruments. 90 of 90 tissues tested scored equivalently.

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