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

REF 790-4373

05479266001

IVD Σ 50

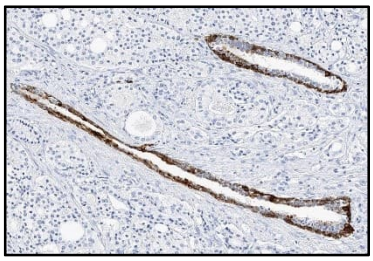

Figure 1. CONFIRM anti-Keratin (34βE12) antibody staining basal cells in prostate tissue with no staining in the adjacent prostatic adenocarcinoma.

INTENDED USE

CONFIRM anti-Keratin (34βE12) Mouse Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of cytokeratin 5 by light microscopy in sections of formalin-fixed, paraffin-embedded tissue stained on a BenchMark IHC/ISH instrument. This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information and proper controls.

This antibody is intended for in vitro diagnostic use.

SUMMARY AND EXPLANATION

CONFIRM anti-Keratin (34βE12) Mouse Monoclonal Primary Antibody (CONFIRM anti-Keratin (34βE12) antibody) recognizes cytokeratin 5, and by extension its in vivo partner cytokeratin 14, as well as cytokeratin 1 and its partner cytokeratin 10.[1,2] The expression of individual cytokeratins is characteristic of particular organs and is maintained in neoplasms originating from those organs. Thus, cytokeratins provide neoplastic fingerprints and are used in diagnostic pathology for the identification of tumors from different tissues.[1,3]

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.[2] Filaments containing cytokeratin proteins are characteristic of epithelial cells.[4] Clone 34βE12 has been characterized as identifying the high molecular weight cytokeratin 5 (62 kDa).[2,3] Cytokeratin 5 is expressed in basal cells and not luminal cells of the prostate and is typically absent in neoplasms that have breached the basal membrane, eliminating the presence of prostatic basal cells.[2,5,6] Clone 34βE12 reacts with basal cells in normal epithelia of the prostate.[3] The detection of cytokeratin 5 by immunohistochemistry (IHC) with CONFIRM anti-Keratin (34βE12) antibody may be used for the detection of basal cells to aid in the differentiation of benign and malignant prostate lesions.

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-Keratin (34βE12) antibody binds to keratin proteins in formalin fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

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

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

The antibody is diluted in Tris-HCl with carrier protein, and 0.10% ProClin 300, a preservative.

Specific antibody concentration is approximately 1.4 µg/mL. There is no known non-specific antibody reactivity observed in this product.

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

CONFIRM anti-Keratin (34βE12) antibody is a mouse monoclonal antibody produced as ascites material.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation, Quality Control Procedures, Troubleshooting, Interpretation of Results, and 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 method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001)
4. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
5. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. General purpose laboratory equipment
15. BenchMark IHC/ISH Instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°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 FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.[7] Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

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. Do not use beyond the specified number of tests.
4. 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.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[8,9]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.

9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9,reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to the tables 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 User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-4373.

Table 2. Recommended staining protocol for CONFIRM anti-Keratin (34βE12) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Mild	CC1, Mild	ULTRA CC1, 36 minutes (Mild), 95°C
Antibody (Primary)	28 minutes, 37°C	32 minutes, 37°C	32 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

Table 3. Recommended staining protocol for CONFIRM anti-Keratin (34βE12) antibody with OptiView Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 32 minutes	CC1, 32 minutes	ULTRA CC1, 32 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected	Selected
Antibody (Primary)	12 minutes, 37°C	16 minutes, 37°C	16 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

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."[10]

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-Keratin (34βE12) antibody, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

An example of a positive control tissue for CONFIRM anti-Keratin (34βE12) antibody is benign prostate. Basal cells in benign and normal prostate glands should stain positively.

STAINING INTERPRETATION / EXPECTED RESULTS

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

SPECIFIC LIMITATIONS

Presence of cytoplasmic staining may be observed which indicates positive staining but not necessarily the presence of basal cells in tissues other than prostate or tumors of the prostate. Interpretation by a qualified pathologist should be in conjunction with histological examination, relevant clinical information, and proper controls.

OptiView detection system is generally more sensitive than *ultra*View detection system. The user must validate the results obtained with this reagent and detection systems.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 4. Sensitivity/Specificity of CONFIRM anti-Keratin (34βE12) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Stomach	3/3
Cerebellum	0/3	Small intestine	1/3
Adrenal gland	0/3	Colon	3/3
Ovary	4/4	Liver [d]	0/3
Pancreas	3/3	Salivary gland	3/3
Lymph node	0/3	Larynx	2/2
Pituitary gland	3/3	Kidney	3/3
Testis	0/3	Prostate [e]	7/7
Thyroid	0/3	Bladder	4/4
Breast [a]	3/6	Parathyroid gland	0/3
Spleen	0/3	Endometrium	3/3
Tonsil [b]	6/6	Cervix	3/3
Thymus [c]	3/3	Skeletal muscle	0/3
Bone marrow	0/3	Skin	3/3
Lung	3/3	Nerve	0/4
Heart	0/3	Mesothelium	3/3
Esophagus	3/3	Appendix	1/1

[a] Ductal epithelial and myoepithelial cells staining; tissues evaluated included normal and benign breast. [b] Squamous epithelium. [c] Epithelial cells. [d] Hepatocytes. [e] Tissues evaluated included normal and hyperplastic prostate.

Table 5. Sensitivity/Specificity of CONFIRM anti-Keratin (34βE12) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	1/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous carcinoma (Ovary)	1/1
Adult granulosa cell tumor (Ovary)	0/1
Teratoma (Ovary)	1/1
Neuroendocrine carcinoma (Pancreas)	0/1
Ductal Adenocarcinoma (Pancreas)	1/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	1/1
Follicular carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (DCIS) (Breast) [a]	5/5
Invasive ductal carcinoma (Breast) [a]	17/18
Invasive lobular carcinoma (Breast) [a]	1/1
Fibroadenoma (Breast) [a]	0/2
Diffuse large B-cell lymphoma (Spleen)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	1/1
Adenocarcinoma (Lung)	1/1
Squamous cell carcinoma (Esophagus)	1/1
Adenocarcinoma (Esophagus)	0/1
Adenocarcinoma (Stomach)	1/1
Gastrointestinal stromal tumor (GIST) (Stomach)	0/1
Adenocarcinoma (Small intestine)	1/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	0/1
Adenocarcinoma (Colon)	0/1
Adenosquamous carcinoma (Colon)	1/1
Hepatocellular carcinoma (Liver)	0/1
Cholangiocarcinoma (Liver)	1/1
Renal cell carcinoma (Kidney)	0/1
Papillary adenoma (Kidney)	1/1
Adenocarcinoma (Prostate)	1/85
Leiomyoma (Prostate)	0/1
Sarcomatoid carcinoma (Prostate)	0/1
Leiomyoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	1/1
Adenocarcinoma (Uterus)	1/1
Leiomyosarcoma (Abdomen)	0/1
Squamous cell carcinoma (Cervix)	1/1
Adenocarcinoma (Cervix)	1/1
Rhabdomyosarcoma (Muscle)	0/1
Myxoma (Heart)	0/1
Basal cell carcinoma (Skin)	1/1
Squamous cell carcinoma (Skin)	1/1
Melanoma (Skin)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Follicular lymphoma (Lymph node)	0/1
Hodgkin lymphoma (Lymph node)	0/1
Urothelial Carcinoma (Bladder)	1/1
Squamous cell carcinoma (Bladder)	1/1
Multiply myeloma (Bone marrow)	0/1
Adenoma (Adrenal gland)	0/1
Pheochromocytoma (Adrenal gland)	0/1
Pleomorphic adenoma (Salivary gland)	1/1

Pathology	# positive / total cases
Warthin tumor (Salivary gland)	1/1
Well-differentiated neuroendocrine tumor (Appendix)	0/1
Malignant peripheral nerve sheath tumor (MPNST) (Prostate)	0/1
Schwannoma (Spinal cord)	0/1
Mesothelioma (Stomach)	1/1
Solitary fibrous tumor (Pleura)	0/1
Angiosarcoma (Soft tissue)	0/1
Adenocarcinoma (Sinus)	1/1
Squamous cell carcinoma (Sinus)	1/1
Fibrosarcoma (Abdomen)	0/1
Liposarcoma (Soft tissue)	0/1

[a] Myoepithelial cells

Precision

Precision studies for CONFIRM anti-Keratin (34βE12) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX, BenchMark XT, BenchMark ULTRA instrument.
- Between platform precision between the BenchMark GX, BenchMark XT, BenchMark ULTRA instrument.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of CONFIRM anti-Keratin (34βE12) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

REFERENCES

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information):

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
E	Updates to Warnings and Precautions section. Updated to current template.

INTELLECTUAL PROPERTY

For USA: Rx only

CONTACT INFORMATION

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

www.roche.com

EC REP

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany
+800 5505 6606

CE 0123