Anti-Pan Keratin (AE1/AE3/PCK26) Primary Antibody

REF 760-2595 Σ 50

05267145001

REF 760-2135 Σ 250

05266840001

IVD

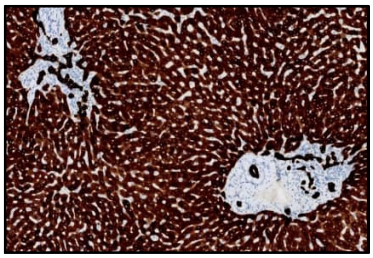

Figure 1. Normal liver stained with Anti-Pan Keratin (AE1/AE3/PCK26) antibody

INTENDED USE

Anti-Pan Keratin (AE1/AE3/PCK26) Primary Antibody is an antibody cocktail intended for laboratory use in the qualitative immunohistochemical detection of most acidic cytokeratins and all basic cytokeratins 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 (IVD) use.

SUMMARY AND EXPLANATION

Anti-Pan Keratin (AE1/AE3/PCK26) Primary Antibody (Anti-Pan Keratin (AE1/AE3/PCK26) antibody) is a cocktail of three monoclonal antibody clones – AE1, AE3, and PCK26. The combination of all three clones recognizes most acidic type I cytokeratins and all basic type II cytokeratins.[1,2] Cytokeratins, also known as keratins, are intermediate filaments found on the cytoskeleton of epithelial cells to provide structural support and resist mechanical stress.[2-5] They are divided into acidic and basic categories and occur in pairs in epithelial tissues, with the composition of pairs varying with the epithelial cell type, stage of differentiation, cellular growth environment, and disease state.[2-5] Historically, Pan Keratin antibodies containing only AE1/AE3 clones often under-detect carcinomas derived from the liver and kidney.[1,6] The PCK26 clone was added to the AE1/AE3 cocktail because it detects cytokeratin 8 along with other cytokeratins. Detection of cytokeratin 8 is important because it is one of two keratins expressed in the liver.[1,2,3,6] Additionally, cytokeratin 8 is one of the earliest cytokeratins expressed during epithelial development and its expression is often maintained in epithelial neoplasms.[1,2,3,6] By combining all three clones, a single reagent with a broad spectrum of reactivity with both high and lower molecular weight cytokeratins is obtained. Thus, Anti-Pan Keratin (AE1/AE3/PCK26) antibody detects the majority of carcinoma cases.[2,3,4] In addition to carcinomas, Pan Keratin antibodies can also recognize cases of epithelioid sarcoma, synovial sarcoma, and mesothelioma.[1,7]

The detection of most acidic cytokeratins and all basic cytokeratins by immunohistochemistry (IHC) with the Anti-Pan Keratin (AE1/AE3/PCK26) antibody may be used to aid in the identification of neoplasms of epithelial origin. This antibody may be used as part of a panel of IHC studies. The staining pattern is cytoplasmic.

PRINCIPLE OF THE PROCEDURE

Anti-Pan Keratin (AE1/AE3/PCK26) antibody contains a cocktail of mouse monoclonal antibodies raised against human epidermal keratins.[8] This antibody cocktail reacts with the 56.5kD, 50kD, 48kD, and 40kD cytokeratins of the acidic subfamily and 65-67kD, 64kD, 59kD, 58kD, 56kD, and 52kD cytokeratins of the basic subfamily.[2,3,7] Anti-Pan Keratin (AE1/AE3/PCK26) antibody binds to keratins in formalin-fixed, paraffin-embedded (FFPE) tissue and exhibits a cytoplasmic staining pattern. This antibody cocktail may be visualized using the *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001). Refer to the *ultra*View Universal DAB Detection Kit method sheet for further information.

MATERIAL PROVIDED

Anti-Pan Keratin (AE1/AE3/PCK26) antibody (Cat. No. 760-2595) contains sufficient reagent for 50 tests.

One 5 mL dispenser of Anti-Pan Keratin (AE1/AE3/PCK26) antibody (Cat. No. 760-2595) contains approximately 231.5 µg of a mouse monoclonal antibody cocktail.

Anti-Pan Keratin (AE1/AE3/PCK26) antibody (Cat. No. 760-2135) contains sufficient reagent for 250 tests.

One 25 mL dispenser of Anti-Pan Keratin (AE1/AE3/PCK26) antibody (Cat. No. 760-2135) contains approximately 1.2 mg of a mouse monoclonal antibody cocktail.

The antibody is diluted in phosphate buffered saline containing carrier protein and 0.05% ProClin 300, a preservative.

Specific antibody concentration is approximately 46.3 µg/mL.

Anti-Pan Keratin (AE1/AE3/PCK26) antibody is a mouse monoclonal cocktail; components are produced as ascites material (PCK26) or purified antibody (AE1 and AE3).

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and 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 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. Protease 3 (Cat. No. 760-2020 / 05266718001)
6. VENTANA Antibody Diluent with Casein (Cat. No. 760-219 / 06440002001)
7. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
8. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
9. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
10. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
11. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
12. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
13. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
14. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
15. General purpose laboratory equipment
16. 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 the 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.[9] 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. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.
5. 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.
6. 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.
7. 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.[10,11]
8. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
9. Avoid microbial contamination of reagents as it may cause incorrect results.
10. 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.
11. Consult local and/or state authorities with regard to recommended method of disposal.
12. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
13. 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.
	P261	Avoid breathing mist or vapours.
	P272	Contaminated work clothing should not be allowed out of the workplace.
	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 Table 2 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 760-2595.

Table 2. Recommended staining protocol for Anti-Pan Keratin (AE1/AE3/PCK26) 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, 95°C
Enzyme (Protease)	Protease 3, 4 minutes		
Antibody (Primary)	4 minutes, 37°C	8 minutes, 37°C	8 minutes, 36°C
ultraBlock step using VENTANA Antibody Diluent with Casein [b]	4 minutes		
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.

[b] Use of VENTANA Antibody Diluent with Casein at the ultraBlock step is recommended to reduce staining on smooth muscle.

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."[12]

NEGATIVE REAGENT CONTROL

In addition to staining with the Anti-Pan Keratin (AE1/AE3/PCK26) 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 this antibody is the epithelium of skin.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for Anti-Pan Keratin (AE1/AE3/PCK26) antibody is cytoplasmic.

SPECIFIC LIMITATIONS

Occasionally, stromal elements surrounding heavily-stained tissue and/or cells will show immunoreactivity. Specific off-target staining of smooth muscle, reticulum cells in lymphoid tissues, and endothelial cells was noted with this antibody cocktail, which in most cases was mild to moderate, but in some cases focally severe. The use of a blocking reagent (VENTANA Antibody Diluent with Casein) reduced, but did not eliminate, off-target staining without compromising the specific reactivity and is therefore recommended. Residual off-target staining should not interfere with interpretation of the stain.

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 3. Sensitivity/Specificity of Anti-Pan Keratin (AE1/AE3/PCK26) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Esophagus	3/3
Cerebellum	0/3	Stomach	3/3
Adrenal gland [a]	3/3	Small intestine	3/3
Ovary [b]	3/3	Colon	10/10
Pancreas	3/3	Liver [e]	11/11
Lymph node	0/3	Salivary gland	3/3
Pituitary gland	3/3	Kidney	34/34
Testis	0/3	Prostate [f]	12/12
Thyroid	3/3	Bladder	3/3
Breast [b]	3/3	Parathyroid gland	3/3
Spleen [c]	1/3	Cervix	3/3
Tonsil [d]	3/3	Skeletal muscle	0/3
Thymus [b]	3/3	Skin	3/3
Bone marrow	0/3	Nerve	0/3
Lung	6/6	Mesothelium	3/3
Heart	0/3		

[a] Cortex, [b] Epithelial cells, [c] Off-target staining of littoral cells, [d] Squamous epithelium, [e] Hepatocytes and bile ducts, [f] Tissues evaluated include normal and hyperplastic prostate

Table 4. Sensitivity/Specificity of Anti-Pan Keratin (AE1/AE3/PCK26) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum) [a]	1/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous adenocarcinoma (Ovary)	1/1
Adenocarcinoma (Ovary)	1/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	1/1
Seminoma (Testis) [b]	2/2
Medullary carcinoma (Thyroid)	1/1
Papillary carcinoma (Thyroid)	1/1
Ductal carcinoma in situ (Breast)	4/4
Medullary carcinoma (Breast)	2/2
Invasive papillary carcinoma (Breast)	1/1
Mucinous carcinoma (Breast)	3/3
Invasive ductal carcinoma (Breast)	11/11
Invasive lobular carcinoma (Breast)	3/3
Paget disease (Breast)	3/3
B-cell lymphoma, NOS	0/3
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	11/11
Adenocarcinoma (Lung)	10/10
Large cell carcinoma (Lung)	1/1
Neuroendocrine carcinoma (Esophagus)	1/1
Adenocarcinoma (Esophagus)	1/1
Signet-ring cell carcinoma	8/8
Adenocarcinoma (Small intestine)	1/1
Adenocarcinoma (Colorectum)	35/35
Gastrointestinal stromal tumor (GIST)	0/3
Hepatocellular carcinoma (Liver)	35/36
Cholangiocarcinoma	5/5
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	11/11
Papillary carcinoma (Kidney)	10/11
Chromophobe carcinoma (Kidney)	10/10
Adenocarcinoma (Prostate)	16/16
Leiomyoma	0/2
Adenocarcinoma (Uterus)	1/1
Clear cell carcinoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	2/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1
Basal cell carcinoma (Skin)	1/1
Squamous cell carcinoma (Skin)	1/1
Neurofibroma (Lumbar)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Mesothelioma (Peritoneum)	1/1
Hodgkin lymphoma (Lymph node)	0/1

Pathology	# positive / total cases
Anaplastic large cell lymphoma (Lymph node)	0/1
Urothelial carcinoma (Bladder)	1/1
Leiomyosarcoma	0/2
Osteosarcoma (Bone)	0/1
Spindle cell rhabdomyosarcoma	0/1

[a] Positivity may be due to cross-reactivity of the AE1/AE3 antibodies with glial fibrillary acidic protein (GFAP), leading to aberrant staining of glial tumors[13,14]

[b] One case had appropriate sparse staining

Precision

Precision studies for Anti-Pan Keratin (AE1/AE3/PCK26) 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, and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark GX BenchMark XT and 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 Anti-Pan Keratin (AE1/AE3/PCK26) 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 definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
H	Updates to Materials Required but not Provided, Warnings and Precautions section.

INTELLECTUAL PROPERTY

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