

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

**REF** 760-2595

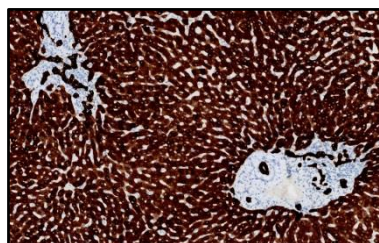
05267145001

**IVD**  50

**REF** 760-2135

05266840001

**IVD**  250



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

qualitative staining in sections of formalin-fixed, paraffin-embedded tissue.

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

Cytokeratins are polypeptide chains which form structural proteins called intermediate filaments comprising the epithelial cell cytoskeleton. Cytokeratins are divided into acidic and basic subfamilies. They occur in pairs in epithelial tissues, the composition of pairs varying with the epithelial cell type, stage of differentiation, cellular growth environment, and disease state. Nineteen different forms of cytokeratin have been characterized by their molecular weight and isoelectric pH in both normal and malignant epithelial cell lines.<sup>1,2,3</sup> Low molecular weight cytokeratins (40-54kD) are frequently expressed in non-stratified columnar epithelium, whereas high molecular weight cytokeratins (48-67kD) are frequently expressed in stratified epithelium.<sup>4</sup> Monoclonal antibodies against various cytokeratins may be useful in determining the origin of poorly differentiated tumors.<sup>5</sup> As cytokeratin peptides are associated with epithelial cells, these peptides are clinically important markers to aid in distinguishing carcinomas from malignant tumors of non-epithelial origin such as lymphomas and sarcomas.<sup>6,7</sup> The identification of cytokeratin has gained increasing importance in immunopathology. A diagnostic algorithm for the use of cytokeratin in the classification of tumors is presented in Immunomicroscopy: A Diagnostic Tool for the Surgical Pathologist, Chapter 14.<sup>1</sup> If keratin reactivity is lacking and a high index of suspicion for carcinoma exists, further immunohistochemical studies should be pursued,<sup>5</sup> as unexpected antigen expression or loss of expression may occur, especially in neoplasms. With the principle exceptions of epithelioid sarcoma, synovial sarcoma, and mesothelioma, anti-keratin antibodies appear to recognize only epithelial neoplasms.<sup>8</sup> Occasionally stromal elements surrounding heavily stained tissue and/or cells will show immunoreactivity.

### PRINCIPLE OF THE PROCEDURE

Anti-Pan Keratin (AE1/AE3/PCK26) recognizes most of the acidic and all of the basic cytokeratins, making it a useful stain for nearly all epithelial tissues and their tumors. Anti-Pan Keratin (AE1/AE3/PCK26) contains a cocktail of mouse monoclonal antibodies raised against human epidermal keratins as reported by Woodcock-Mitchell, et al.<sup>9</sup> This antibody cocktail reacts with the 56.5kD, 50kD, 50'kD, 48kD, and 40kD cytokeratins of the acidic subfamily and 65-67kD, 64kD, 59kD, 58kD, 56kD, and 52kD cytokeratins of the basic subfamily.<sup>9,10,11,12,13</sup> Anti-Pan Keratin (AE1/AE3/PCK26) binds to keratins in formalin-

fixed, paraffin-embedded tissue and exhibits a cytoplasmic staining pattern. This antibody cocktail may be visualized using the *ultraView* Universal DAB Detection Kit (Cat. No. 760-500/05269806001). Refer to the *ultraView* Universal DAB Detection Kit for further information

### REAGENT PROVIDED

Anti-Pan Keratin (AE1/AE3/PCK26) (760-2595) contains sufficient reagent for 50 tests.

One 5 mL dispenser of Anti-Pan Keratin (AE1/AE3/PCK26) (760-2595) contains approximately 231.5 µg of a mouse monoclonal antibody cocktail.

Anti-Pan Keratin (AE1/AE3/PCK26) (760-2135) contains sufficient reagent for 250 tests.

One 25 mL dispenser of Anti-Pan Keratin (AE1/AE3/PCK26) (760-2135) contains approximately 1.6 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.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 46.3 µg/mL.

Anti-Pan Keratin (AE1/AE3/PCK26) 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 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 2-8°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, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA detection kits and the VENTANA BenchMark ULTRA, BenchMark XT, BenchMark GX automated slide stainers. The recommended tissue fixative is 10% neutral buffered formalin.<sup>14</sup> 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. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
6. Avoid microbial contamination of reagents as it may cause incorrect results.
7. Consult local and/or state authorities with regard to recommended method of disposal.
8. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Risk Phrase Guide located at [www.ventana.com](http://www.ventana.com).

## STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on the VENTANA BenchMark ULTRA, BenchMark XT and BenchMark GX 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 instruments Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

**Table 1.** Recommended Staining Protocol for Anti-Pan Keratin (AE1/AE3/PCK26) with *ultraView* Universal DAB Detection Kit on a BenchMark ULTRA instrument, BenchMark XT instrument or BenchMark GX instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Mild
Enzyme (Protease)	Protease 3, 4 minutes
Antibody (Primary)	BenchMark ULTRA instrument 8 minutes, 36°C BenchMark XT instrument 8 minutes, 37°C BenchMark GX instrument 4 minutes, 37°C
ultraBlock	*VENTANA Antibody Diluent with Casein, 4 minutes
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

\*Use of VENTANA Antibody Diluent with Casein (Cat. No. 760-219/06440002001) 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".<sup>15</sup>

## POSITIVE TISSUE CONTROL

An example of a positive control tissue for this antibody is skin.

## STAINING INTERPRETATION

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

## SPECIFIC LIMITATIONS

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.

## PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity and repeatability were conducted with the *ultraView* Universal DAB Detection Kit and the results are listed in Table 2 and Table 3.

### Specificity

**Table 2.** Specificity of Anti-Pan Keratin (AE1/AE3/PCK26) was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus (epithelial components)	3/3
Cerebellum	0/3	Myeloid (bone marrow)	0/3
Adrenal gland (cortex)	2/2	Lung	7/7
Ovary (epithelial cells)	3/3	Heart (cardiac muscle)	0/3
Pancreas	2/2	Esophagus	3/3
Lymph node	0/3	Stomach	3/3
Adenohypophysis	3/3	Small intestine	3/3
Testis	0/3	Colon	10/10
Thyroid	3/3	Liver (hepatocytes and bile ducts)	11/11
Breast (epithelium)	2/2	Tongue (epithelial cells)	3/3
Spleen <sup>a</sup>	1/3	Kidney	34/34
Tonsil (squamous epithelium)	3/3	Prostate	5/5
Bladder	3/3	Parathyroid gland	3/3
Skeletal muscle (tongue)	0/1	Cervix	2/2
Peripheral nerve	0/3	Skin	3/3
Mesothelium (cardiac pericardium)	2/2		

<sup>a</sup>: Off target staining of littoral cells

### Sensitivity

**Table 3.** Sensitivity of Anti-Pan Keratin (AE1/AE3/PCK26) was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma <sup>a</sup>	1/1
Atypical meningioma	0/1
Anaplastic ependymoma	0/1
Oligodendroglioma	0/1
Ovarian serous adenocarcinoma	1/1
Ovarian adenocarcinoma	1/1
Pancreatic endocrine neoplasm (Islet cell tumor)	0/1
Pancreatic adenocarcinoma	1/1
Seminoma <sup>b</sup>	2/2
Thyroid medullary carcinoma	1/1
Thyroid papillary carcinoma	1/1
Breast intraductal carcinoma	4/4
Breast medullary carcinoma	2/2

Pathology	# positive / total cases
Breast papillary carcinoma	1/1
Breast mucinous carcinoma	3/3
Breast invasive ductal carcinoma	11/11
Breast invasive lobular carcinoma	3/3
Paget's disease	3/3
Diffuse large B-cell lymphoma	0/3
Lung small cell undifferentiated carcinoma	0/1
Lung squamous cell carcinoma	11/11
Lung adenocarcinoma	10/10
Lung large cell undifferentiated carcinoma	1/1
Esophageal neuroendocrine carcinoma	1/1
Esophageal adenocarcinoma	1/1
Signet-ring cell carcinoma	8/8
Small intestine adenocarcinoma	1/1
Colorectal adenocarcinoma	35/35
GIST	0/3
Hepatocellular carcinoma	35/36
Cholangiocarcinoma	5/5
Hepatoblastoma	0/1
Renal clear cell carcinoma	11/11
Renal papillary carcinoma	10/11
Renal chromophobe carcinoma	10/10
Prostatic adenocarcinoma	16/16
Prostatic glandular hyperplasia	7/7
Leiomyoma	0/2
Endometrial adenocarcinoma	1/1
Endometrial clear cell carcinoma	1/1
Cervical squamous cell carcinoma	2/2
Embryonal rhabdomyosarcoma	0/1
Basal cell carcinoma	1/1
Skin squamous cell carcinoma	1/1
Neurofibroma	0/1
Neuroblastoma	0/1
Mesothelioma	1/1
Hodgkin lymphoma	0/1
Anaplastic large cell lymphoma	0/1
Urothelial carcinoma	1/1

Pathology	# positive / total cases
Low grade leiomyosarcoma	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Intermediate grade leiomyosarcoma	0/1

<sup>a</sup>: 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<sup>16</sup>

<sup>b</sup>: One case had appropriate sparse staining

#### Repeatability

Repeatability studies for Anti-Pan Keratin (AE1/AE3/PCK26) were completed to demonstrate:

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

All studies met their acceptance criteria.

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