


anti-Cytokeratin 5/6 (D5/16B4) Mouse Monoclonal Primary Antibody

REF 790-4554

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IVD  50

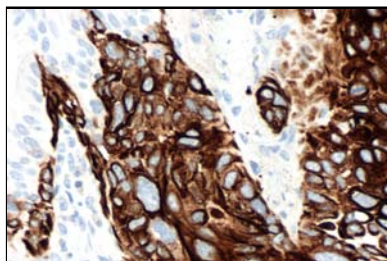


Figure 1. anti-Cytokeratin 5/6 (D5/16B4) staining of lung squamous cell carcinoma

malignant mesothelioma from lung adenocarcinomas. The detection of cytokeratin 5 in myoepithelial cells with this antibody may also be used to aid in the determination of breast and prostate malignancies. The antibody is intended for 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

Anti-Cytokeratin 5/6 (D5/16B4) is a mouse monoclonal antibody produced in response to immunizing human cytokeratin 5 protein; however, it also reacts strongly with cytokeratin 6 and weakly with cytokeratin 4. Cytokeratin 5 is expressed in various epithelia and mesothelial cells, while cytokeratin 6 is expressed by proliferating squamous epithelium.¹ Detection of cytokeratin 5 in mesothelial cells as opposed to adenocarcinomas, may aid in the differentiation of malignant epithelioid mesothelioma from lung adenocarcinomas.^{2,3,4} The reactivity of anti-Cytokeratin 5/6 (D5/16B4) with normal prostatic basal cells and breast myoepithelial cells may assist in distinguishing normal from malignant prostate and breast tissue.^{5,6} In addition, the majority of squamous cell carcinoma demonstrate positivity with this antibody.⁷

REAGENT PROVIDED

Anti-Cytokeratin 5/6 (D5/16B4) contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-Cytokeratin 5/6 (D5/16B4) contains approximately 52 µg of a mouse monoclonal antibody.

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

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 10.4 µg/mL. There is no known non-specific antibody reactivity observed in this product.

Anti-Cytokeratin 5/6 (D5/16B4) is a monoclonal antibody produced as 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 (*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 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 a Ventana BenchMark Series automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.⁸ 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

- For *in vitro* diagnostic (IVD) use.
- ProClin 300 is used as a preservative in this solution. 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.
- Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
- Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- Avoid microbial contamination of reagents as it may cause incorrect results.
- Consult local and/or state authorities with regard to recommended method of disposal.
- Refer to the MSDS for additional precautions and warnings.

STAINING PROCEDURE

Ventana primary antibodies have been developed for use on a Ventana BenchMark Series automated slide stainer in combination with Ventana detection kits and accessories. A recommended staining protocol for the BenchMark XT and BenchMark ULTRA instrument with *ultraView* Universal DAB Detection Kit is listed 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 kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for anti-Cytokeratin 5/6 (D5/16B4) with *ultraView* Universal DAB Detection Kit on a BenchMark XT 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 Approximately 16 Minutes, 37°C BenchMark ULTRA instrument Approximately 20 Minutes, 36°C
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bluing, 4 Minutes

Table 2. Recommended Staining Protocol for anti-Cytokeratin 5/6 (D5/16B4) with M/IEW DAB Detection Kit on a BenchMark XT instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Enzyme (Protease)	None required
Antibody (Primary)	BenchMark XT instrument Approximately 32 Minutes, 37°C
Counterstain	Hematoxylin II, 4 Minutes
Post Counterstain	Bluing, 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".⁹

POSITIVE TISSUE CONTROL

Examples of positive control tissues for this antibody are normal prostate and normal tonsil.

STAINING INTERPRETATION

The cellular staining pattern for anti-Cytokeratin 5/6 (D5/16B4) is cytoplasmic.

SPECIFIC LIMITATIONS

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

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and reproducibility were conducted using anti-Cytokeratin 5/6 (D5/16B4) with *ultra*View Universal DAB Detection Kit on BenchMark XT and BenchMark ULTRA instruments.

Specificity

Table 3. Specificity of anti-Cytokeratin 5/6 (D5/16B4) was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	3/3
Cerebellum	0/3	Myeloid (bone marrow)	0/3
Adrenal gland	0/3	Lung	2/6
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus	3/3
Parathyroid gland	0/3	Stomach	0/3
Hypophysis	0/3	Small intestine	0/3
Testis	0/3	Colon	0/3
Thyroid	0/3	Liver	0/3
Breast	41/51	Salivary gland	0/3
Spleen	0/3	Kidney	0/2

Tissue	# positive / total cases	Tissue	# positive / total cases
Tonsil	3/3	Prostate	22/23
Endometrium	0/3	Cervix	3/3
Skeletal muscle	0/3	Skin	3/3
Nerve (sparse)	0/3	Mesothelium and lung	0/2

Sensitivity

Table 4. Sensitivity of anti-Cytokeratin 5/6 (D5/16B4) was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	0/1
Malignant ependymoma	0/1
Malignant oligodendroglioma	0/1
Serous papillary adenocarcinoma	0/1
Mucinous papillary adenocarcinoma	0/1
Islet cell carcinoma	0/1
Pancreatic adenocarcinoma	0/1
Seminoma	0/1
Embryonal carcinoma	0/1
Medullary carcinoma	0/1
Papillary carcinoma	0/1
Breast intraductal carcinoma	0/3
Breast lobular carcinoma in situ	0/1
Breast invasive ductal carcinoma	0/1
Breast infiltrating duct carcinoma	2/18
Diffuse B-cell lymphoma	0/1
Lung small cell undifferentiated carcinoma	0/1
Lung squamous cell carcinoma	42/50
Lung adenocarcinoma	0/15
Lung papillary carcinoma	0/1
Lung clear cell carcinoma	0/1
Lung carcinoid	0/1
Lung neuroendocrine tumor	0/4
Lung bronchioloalveolar carcinoma	0/11
Lung adenosquamous carcinoma	6/11
Lung undifferentiated carcinoma	4/5
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	1/41

Pathology	# positive / total cases
Prostatic transitional cell carcinoma	1/1
Leiomyoma	0/1
Endometrial adenocarcinoma	1/1
Endometrial clear cell carcinoma	0/1
Uterine squamous cell carcinoma	1/2
Embryonal rhabdomyosarcoma	0/1
Anal malignant melanoma	0/1
Basal cell carcinoma	1/1
Squamous cell carcinoma	0/1
Neurofibroma	0/1
Retroperitoneal neuroblastoma	0/1
Malignant mesothelioma	0/1
Diffuse malignant lymphoma	0/3
Hodgkin's lymphoma	0/1
Bladder transitional cell carcinoma	1/1
Low grade leiomyosarcoma	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Intermediate grade leiomyosarcoma	0/1
Malignant epithelial mesothelioma	12/25
Malignant mixed mesothelioma	0/6
Malignant spindled mesothelioma	0/6
Benign mixed mesothelioma	0/2

Repeatability

Repeatability studies for anti-Cytokeratin 5/6 (D5/16B4) 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 and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

Compatibility Results

Anti-Cytokeratin 5/6 (D5/16B4) has demonstrated compatibility with *ultraView* Universal DAB Detection Kit and BenchMark XT and BenchMark ULTRA instruments.

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