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

REF 790-4554

06478441001

IVD Σ 50

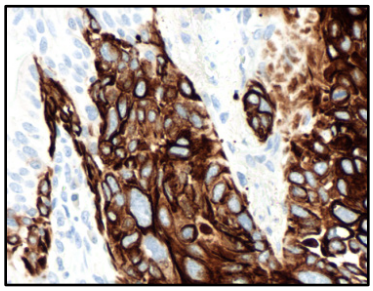

Figure 1. anti-Cytokeratin 5/6 (D5/16B4) antibody staining of lung squamous cell carcinoma.

INTENDED USE

Anti-Cytokeratin 5/6 (D5/16B4) 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 (IVD) use.

SUMMARY AND EXPLANATION

Anti-Cytokeratin 5/6 (D5/16B4) Mouse Monoclonal Primary Antibody (anti-Cytokeratin 5/6 (D5/16B4) antibody) is a mouse monoclonal antibody raised for the detection of cytokeratin (CK) 5. CK5 is a type II CK (high molecular weight, 62 kDa) expressed in the basal and myoepithelial cells of various epithelia and glandular structures including the bronchi, prostate and breast.[1,2]

CK5 is expressed in the basal epithelium of the lung and its overexpression is an indicator of malignant squamous differentiation in pulmonary lesions.[1,3] Malignant squamous differentiation is characteristic of the squamous cell carcinoma (SCCA) subtype of non-small cell lung cancer (NSCLC).[4,5] The immunohistochemistry (IHC)-based detection of CK5 with anti-Cytokeratin 5/6 (D5/16B4) antibody can be used to aid in the distinction between pulmonary SCCA and pulmonary adenocarcinoma (ADC).

In addition, CK5 is expressed in mesothelial cells of the pulmonary pleura and is overexpressed in epithelioid malignant mesothelioma (MM).[6] CK5 is consistently absent in pulmonary ADC that confounds the diagnosis of epithelioid MM.[6,7] Thus, detection of CK5 with anti-Cytokeratin 5/6 (D5/16B4) antibody can be used to aid in the differential diagnosis of epithelioid MM from lung ADC.

CK5 is expressed in prostatic basal cells and the loss of CK5 expression is indicative of neoplasms that have breached the basal membrane.[1,8] The detection of CK5 in basal cells is a feature of normal and benign prostatic processes and the absence of CK5 is indicative of prostate adenocarcinoma.[9,10] Thus, detection of CK5 in prostatic basal cells with anti-Cytokeratin 5/6 (D5/16B4) antibody may be used to aid in the differentiation of benign and malignant prostate lesions.

Additionally, CK5 is expressed in myoepithelial cells (MECs) in normal breast tissue and benign processes.[11,12] The absence of MECs is a diagnostic feature of invasive processes.[13-15] In cases where the MEC layer is difficult to evaluate via histology alone, ancillary IHC assays for MEC markers can be used.[13-15] Several MEC markers exist and recommendations include use of IHC studies for at least two markers to demonstrate the presence or absence of myoepithelial cells.[13-15]The detection of CK5 in breast MECs with anti-Cytokeratin 5/6 (D5/16B4) antibody may be used to aid in distinguishing non-invasive from invasive breast neoplasms.

The antibody clone D5/16B4, raised for the detection of CK5, is also reactive to CK6, another type II CK (60 kDa) that shares 86% sequence homology to CK5. CK6 is expressed in hyperproliferative keratinocytes and is not used as a basal cell marker.[1] The antibody D5/16B4 is often referred to as CK5/6 antibody in the pathology literature.

This antibody may be used as part of a panel of IHC studies.

PRINCIPLE OF THE PROCEDURE

The anti-Cytokeratin 5/6 (D5/16B4) antibody binds cytokeratin 5 and cytokeratin 6 in formalin-fixed, paraffin-embedded (FFPE) tissue sections and exhibits a cytoplasmic staining pattern. 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

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

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

The antibody is diluted in a phosphate buffered saline containing carrier protein and 0.10% ProClin 300, a preservative.

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) antibody is a monoclonal antibody produced as cell culture supernatant.

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 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.

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. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
8. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
9. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
10. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. Permanent mounting medium
14. Cover glass
15. Automated coverslipper
16. General purpose laboratory equipment
17. 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.[16] 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 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.
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.[17,18]
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 dialog.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, a 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 790-4554.

Table 2. Recommended staining protocol for anti-Cytokeratin 5/6 (D5/16B4) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard	ULTRA Cell Conditioning 1 64 minutes, 95 °C
Antibody (Primary)	16 minutes, 37 °C	20 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."[19]

NEGATIVE REAGENT CONTROL

In addition to staining with anti-Cytokeratin 5/6 (D5/16B4) 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.

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

STAINING INTERPRETATION / EXPECTED RESULTS

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

SPECIFIC LIMITATIONS

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 specificity, sensitivity, precision, and method comparison were conducted and the results are listed below.

Sensitivity and Specificity

Table 3. Sensitivity/Specificity of anti-Cytokeratin 5/6 (D5/16B4) antibody was determined by testing FFPE 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

Tissue	# positive / total cases	Tissue	# positive / total cases
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus	3/3
Parathyroid gland	0/3	Stomach	0/3
Hypophysis (Pituitary)	0/3	Small intestine	0/3
Testis	0/3	Colon	0/3
Thyroid	0/3	Liver	0/3
Breast	41/51*	Salivary gland	3/3
Spleen	0/3	Kidney	1/3
Tonsil	3/3	Prostate	22/23
Endometrium	0/3	Cervix	3/3
Skeletal muscle	0/3	Skin	3/3
Nerve	0/3	Mesothelium and lung	1/3
Bladder	3/3		

* Incomplete or partial staining of MECs in breast tissue may be observed.

Table 4. Sensitivity/Specificity of anti-Cytokeratin 5/6 (D5/16B4) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous carcinoma (Ovary)	0/1
Mucinous carcinoma (Ovary)	0/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/3
Lobular carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	2/19
B-cell lymphoma NOS (Spleen)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	42/50
Adenocarcinoma (Lung)	0/15
Papillary carcinoma (Lung)	0/1
Clear cell carcinoma (Lung)	0/1
Neuroendocrine carcinoma, typical carcinoid tumor (Lung)	0/1
Neuroendocrine carcinoma (Lung)	0/4
Adenocarcinoma in situ (Lung)	0/11
Adenosquamous carcinoma (Lung)	6/11
Undifferentiated carcinoma (Lung)	4/5
Squamous cell carcinoma (Esophagus)	1/1
Adenocarcinoma (Esophagus)	0/1
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Small intestine)	0/1
Gastrointestinal stromal tumor (GIST)	0/3
Adenocarcinoma (Colorectal)	0/2
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	1/41
Urothelial carcinoma (Prostatic urethra)	1/1
Leiomyoma (Uterus)	0/1
Carcinoma (Uterus)	1/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Uterus)	1/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1
Melanoma (Anus)	0/1
Basal cell carcinoma (Skin)	1/1
Squamous cell carcinoma (Skin)	0/1
Neurofibroma (Lumbar)	0/1
Neuroblastoma (Retroperitoneum)	0/1
Mesothelioma	0/1
Lymphoma, NOS (Lymph node)	0/3
Hodgkin lymphoma (Lymph node)	0/1
Urothelial carcinoma (Bladder)	1/1
Leiomyosarcoma (Bladder)	0/2
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma (Peritoneum)	0/1
Epithelioid mesothelioma	12/25
Biphasic mesothelioma	0/6
Sarcomatoid mesothelioma	0/6
Benign mesothelial proliferation	0/2

Precision

Precision studies for anti-Cytokeratin 5/6 (D5/16B4) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark XT instrument.
- Between instrument precision on the BenchMark XT and BenchMark ULTRA instrument.
- Between platform precision between the 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-Cytokeratin 5/6 (D5/16B4) 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

1. Moll R, Divo M, Langbein L. The Human Keratins: Biology and Pathology. Histochem Cell Biol. 2008;129(6):705-733.
2. Purkis PE, Steel JB, Mackenzie IC, et al. Antibody Markers of Basal Cells in Complex Epithelia. J Sci. 1990;97 (Pt 1):39-50
3. Blobel GA, Moll R, Franke WW, et al. Cytokeratins in normal lung and lung carcinomas. I. Adenocarcinomas, squamous cell carcinomas and cultured cell lines. Virchows Archiv B, Cell Pathology Including Molecular Pathology. 1984;45(4):407-429.
4. Kriegsmann K, Cremer M, Zgorzelski C, et al. Agreement of Ck5/6, P40, and P63 Immunoreactivity in Non-Small Cell Lung Cancer. Pathology. 2019
5. Warth A, Muley T, Herpel E, et al. Large-Scale Comparative Analyses of Immunomarkers for Diagnostic Subtyping of Non-Small-Cell Lung Cancer Biopsies. Histopathology. 2012;61(6):1017-1025.
6. Moll R, Dhouailly D, Sun TT. Expression of Keratin 5 as a Distinctive Feature of Epithelial and Biphasic Mesotheliomas. An Immunohistochemical Study Using Monoclonal Antibody Ae14. Virchows Archiv B, Cell Pathol Incl Mol Pathol. 1989;58(2):129-145.
7. Galateau-Salle F, Churg A, Roggli V, et al. The 2015 World Health Organization Classification of Tumors of the Pleura: Advances since the 2004 Classification. J Thorac Oncol. 2016;11(2):142-154.
8. Yang Y, Hao J, Liu X, et al. Differential expression of cytokeratin mRNA and protein in normal prostate, prostatic intraepithelial neoplasia, and invasive carcinoma. Am J Pathol. 1997;150(2):693-704.
9. Abrahams NA, Ormsby AH, Brainard J. Validation of cytokeratin 5/6 as an effective substitute for keratin 903 in the differentiation of benign from malignant glands in prostate needle biopsies. Histopathology. 2002;41(1):35-41
10. Epstein JI, Egevad L, Humphrey PA, et al. Best practices recommendations in the application of immunohistochemistry in the prostate: report from the International Society of Urologic Pathology consensus conference. Am J Surg Pathol. 2014;38(8):e6-e19.
11. Shao MM, Chan SK, Yu AM, et al. Keratin expression in breast cancers. Virchows Archiv. 2012;461(3):313-322.
12. Gusterson BA, Ross DT, Heath VJ, et al. Basal cytokeratins and their relationship to the cellular origin and functional classification of breast cancer. Breast Cancer Res. 2005;7(4):143-148.
13. Zhao L, Yang X, Khan A, et al. Diagnostic Role of Immunohistochemistry in the Evaluation of Breast Pathology Specimens. Arch. Path. Lab. 2014;138(1):16-24.
14. Dewar R, Fadare O, Gilmore H, Gown AM. Best practices in diagnostic immunohistochemistry: myoepithelial markers in breast pathology. Arch. Path. Lab. 2011;135:422-429.
15. Peng Y, Butt YM, Chen B, Zhang X, Tang P. Update on Immunohistochemical Analysis in Breast Lesions. Arch. Path. Lab. 2017;141:1033-1051.
16. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
17. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
18. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
19. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.

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 dialog.roche.com 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 Summary and Explanation, Hazard information, and Sensitivity and Specificity.

INTELLECTUAL PROPERTY

CONTACT INFORMATION

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

www.roche.com

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

CE 0123