

Anti-Cytokeratin 5, 6, clone D5/16B4



Monoclonal Antibody

Cat. # MAB1620

Lot # LV1463904

pack size: 50 µg

Store at 2-8°C

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IH(P), IF	H, M, R	IgG1	N/A	M	Varies	NP_000415

Background

The cytokeratins are a family of intermediate filaments found in almost all epithelial cells. They are divided into four subclasses depending on their patterns of reactivity and electrophoretic characteristics and are also arranged in a numbered series of 20 or more according to molecular weight and isoelectric point (Quinlan et al. 1985). Types 5 and 6 of cytokeratin (CK5/6) are in high frequency in mesothelial cells, as opposed to cells of adenocarcinoma. CK5/6 has been shown to be a reliable marker for mesothelioma and squamous cell carcinoma of the lung and Bowen's disease. It does not react with pulmonary adenocarcinoma.

Presentation

Purified mouse monoclonal IgG1 in buffer containing 0.02 M Phosphate buffer, 0.25 M NaCl, pH 7.6 with 0.1% sodium azide.

Concentration

1 mg/mL

Specificity

Reacts with human cytokeratin 5 on immunoblots of cytoskeletal preparations of epidermis and non-cytokeratinizing epithelium (Abstract: European Symposium of the Biology of the Cytoskeleton, Helsinki, 18-21, 6, 89, 1989) and on tissue sections. Shows reactivity with cytokeratin 6 and weak reactivity with cytokeratin 4 on immunoblots. No cross-reactivity with cytokeratins 1, 7, 8, 10, 13, 14, 18 or 19. MAB1620 recognizes basal cells and a part of the stratum spinosum in the normal pavement epithelium. The antibody may be useful for distinction of low differentiated pavement epithelium carcinoma and adenocarcinoma. The antibody works on formalin fixed tissues.

Species Cross-reactivity

Human. Expected to cross-react with mouse and rat.

Immunogen

Purified cytokeratin 5 (Exp. Cell Res. (1986) 162:114; J. Invest. Dermatology (1987) 88:191).

Molecular Weight

Cytokeratin 4 (59 kDa), Cytokeratin 5 (58 kDa) and Cytokeratin 6 (56 kDa).

Method of Purification

Protein A Purified

Storage and Handling

Stable for 6 months at 2-8°C in undiluted aliquots from date of receipt.

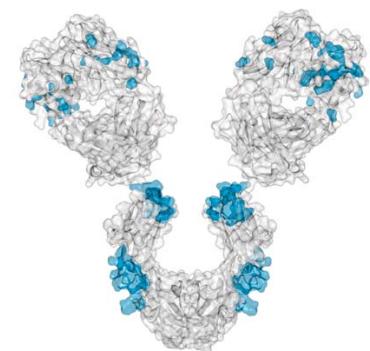
Control

A431 cell lysate, human bladder tumor tissue.

Quality Control Testing

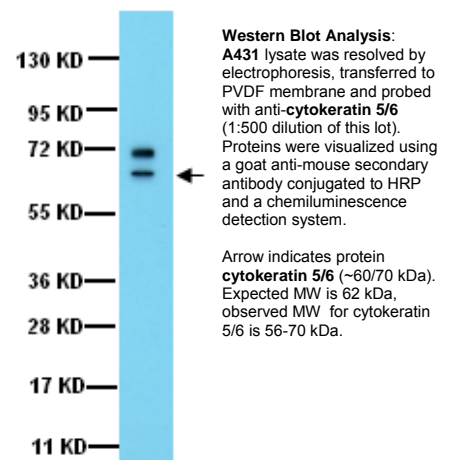
Routinely evaluated by Western Blot on A431 lysates.

Western Blot Analysis: 1:500 dilution of this lot detected **cytokeratin 5/6** on 10 ug of A431 lysates.



References

1. Mikaelian, I., et al. (2004). *Toxicology Pathology*. 32:181-191.
2. EQAS 2002 Evaluation, A. Dodson, ed., UK-NEQAS, (2002). *Immunocytochemistry Journal*. 1(3).
3. Clover, J., et al. (1997). *Histopathology*. 31:140-143.
4. Mischke, D. and Wild, G., (1987). *J. Invest. Dermatology*. 88:191.
5. Wild, G. and Mischke, D., (1986). *Exp. Cell Res.* 162:114.
6. Quinlan, R.A., (1985). *Ann N Y Acad Sci*. 1985;455:282-306.



APPLICATION LEGEND: WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence
IH Immunohistochemistry (Tissue) IH(P) Immunohistochemistry (Paraffin)

SPECIES LEGEND: H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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Additional Research Applications

Immunohistochemistry (frozen or paraffin sections): A 1:50-1:100 dilution from a previous lot was used for paraffin, high-temperature antigen retrieval required (Pressure cooker, Citrate/EDTA buffer pH 6.0, 4 minutes at full pressure).

Immunofluorescence: A previous lot of this antibody was used in Immunofluorescence.

Optimal working dilutions must be determined by end user.

Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 µL or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.

PROTOCOL**Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on cell lysate and transfer the proteins to a PVDF membrane. Wash the PVDF membrane twice with water.
2. Block the blotted PVDF membrane in freshly prepared 5% BSA with 0.05% Tween®-20 for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with the recommended dilution of anti-Cytokeratin 5/6 diluted in freshly prepared 5% BSA for 1 hour at room temperature or overnight with agitation at 2-8°C.
4. Wash the PVDF 3 times with TBST.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG, Catalog # AP124P 1:1000 dilution was used) in 5% milk for 1 hour with agitation at room temperature.
6. Wash the PVDF 3-5 times with TBST.
7. Use Spray and Glow Catalog # 17-373 to visualize results. Use as directed.

APPLICATION NOTES FOR MAB1620

Paraffin Embedded Sections: Formalin-fixed paraffin embedded sections are dewaxed. Before adding the antibody the section should be treated with pronase at 37°C.

Frozen Sections: Ideal frozen sections (4-5 µm) are obtained from shock frozen tissue samples. The frozen sections are air dried and then fixed with acetone for 10 minutes at -20°C. Excess acetone is allowed to evaporate.

- 1) If necessary, block unspecific binding sites by overlaying the sections with 20 µL fetal calf serum for 30 minutes at 37°C in a humid chamber.
- 2) Cover the preparation according to the size of the section with a suitable volume of properly diluted MAB1620 and incubate for one hour at room temperature in a humid chamber.
- 3) Immerse the slides briefly in PBS and wash three times for three minutes each in PBS.
- 4) Wipe slides dry, except the area of the section. Cover the section with 10-20 µL of a solution of anti-Mouse IgG-FITC or anti-Mouse IgG-enzyme and incubate for one hour at room temperature or at 37°C in a humid chamber.
- 5) Wash the slide three times for three minutes each in PBS.

The section should not be allowed to dry out during the steps. If using an enzyme conjugate, the preparation is covered with a substrate solution and incubated at room temperature until a clearly visible color develops.

RELATED PRODUCTS (specific)

cat #	description
MAB3224	■ Anti-Basal Cell Cytokeratin
MAB3412	■ Anti-Cytokeratin AE1/AE3, recognizes acidic & basic cytokeratins, clone AE1/AE3
IHC2025-6	■ IHC Select® Anti-Cytokeratin AE1/AE3 (Pan cytokeratins), prediluted, clone AE1/AE3
IHC2029-6	■ IHC Select® Anti-Cytokeratin High MW, prediluted, clone 34betaE12
IHC2026-6	■ IHC Select® Anti-Cytokeratin Type I, prediluted, clone AE1
IHC2027-6	■ IHC Select® Anti-Cytokeratin Type II, prediluted, clone AE3
04-587	■ Anti-Cytokeratin 5
CBL267	■ Anti-Cytokeratin 5, 14, clone LH8
MAB3228	■ Anti-Cytokeratin 5, 8
IHCR2030-6	■ IHC Select® Anti-Cytokeratin 5, 6, prediluted, clone D5/16B4
IHC2030-6	■ IHC Select® Anti-Cytokeratin 5, 6, prediluted, clone D5/16B4
AP124P	■ Goat anti-Mouse IgG, Peroxidase Conjugated, H+L
AP192F	■ Donkey anti-Mouse IgG, FITC Conjugated

RELATED PRODUCTS (non-specific)

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 µm
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 µm
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0050	■ IMMOBILON WESTERN CHEMILUM HRP SUBSTRATE 50 mL
17-373SP	■ Spray & Glow™ ECL Western Blotting 40 mL
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G
2170	■ CHEMIBLOCKER-1LT
20-200	■ IMMUNOBLOT BLOCKING REAGENT 20G
DAB150-MS	■ IHC SELECT® DAB KIT SINGLE SPECIES-MOUSE 150 TESTS; 1 kit

■ antibodies ■ Multiplex products ■ biotools ■ cell culture ■ enzymes ■ kits ■ proteins/peptides ■ siRNA/cDNA products

Please visit www.millipore.com for additional product information, test data and references

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