Anti-Cytokeratin AE1/AE3, recognizes acidic & basic cytokeratins, clone AE1/AE3

Monoclonal Antibody

Cat. # MAB3412

Lot # 2726803

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS

pack size: 500 µg

Store at 2-8ºC

Certificate of Analysis

Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/ Region	Host Species	Molecular Weight	Accession #
WB, ELISA, IH	H, M, R, Mk, Rb, B, Ch	IgG1	See below	M	40-70 kDa	N/A

Background

Keratins are a group of water-insoluble proteins that form monofilaments, a class of intermediate filament. These filaments form part of the cytoskeletal complex in epidermis and in most other epithelial tissues. Nineteen human epithelial keratins are resolved with two-dimensional gels electrophoresis. These can be divided into acid (pI <5.7) and basic (pI >6.0) subfamilies. The acidic keratins have molecular weights of 56.5, 55, 51, 50, 50', 48, 46, 45, and 40 kDa. The basic keratins have molecular weights of 65-67, 64, 59, 58, 56, and 52 kDa.

Members of the acidic and basic subfamilies are found together in pairs. The composition of keratin pairs varies with the epithelial cell type, stage of differentiation, cellular growth environment, and disease state:

The 56.5/65-67 kDa pair is present in keratinized (differentiated) epidermis. The 55/64 kDa pair is characteristic of normal (corneal-type) epithelial differentiation. The 51/59 kDa pair is characteristic of the stratified squamous epithelial of internal organism such as esophagus and tongue. The 51/58 kDa pair is a keratinocyte marker; the pair is present in almost all stratified epithelia irrespective of the state of cellular stratification. The 48/56 kDa pair is characteristic of hyperproliferative (de-differentiated) keratinocytes. The 45/52 kDa pair and to a lesser extent, the 46/54 kDa pair are characteristic of simple epithelia. The 40 kDa keratin is present in most epithelia except adult epidermis.

Presentation

Anti-keratin AE1/AE3 is supplied in 0.05M borate-buffered saline, 0.15M NaCl, pH 8.2, containing 0.09% sodium azide

Concentration

1.1 mg/mL

Specificity

The stringent, but broad, specificity of pooled AE1/AE3 antibody has made this preparation very useful as a general stain for normal and neoplastic cells of epithelial origin. Anti-Keratin AE1 recognizes the 56.5, 50, 50', 48, and 40 kD keratins of the acidic subfamily. Anti-keratin AE3 recognizes all members of the basic subfamily {65,67,64,59.59,56,52 human basic cytokeratins}.

Epitope/Region

Recognizes acidic & basic cytokeratins

Immunogen

Human epidermal keratins

Molecular Weight

40-70 kDa

Method of Purification

Purified by sodium sulfate precipitation

Storage and Handling

Stable for 1 year at 2-8ºC from date of receipt.

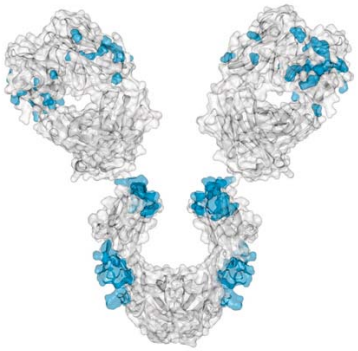
Control

All epithelium-derived tissues & tumors

Quality Control Testing

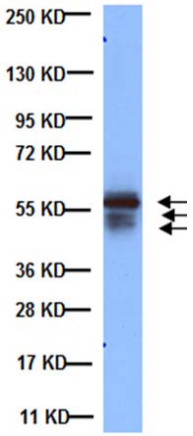
Evaluated by Western Blot on A431 lysates.

Western blot: 1:500 dilution of this antibody detected CYTOKERATIN AE1/AE3 on 10 µg of A431 lysates.



References

1. Woodcock-Mitchel, J. and Sun, T.T. (1982). *Cell*. 30:361.
2. Tseng, D.C.G., Jarvinen, M.J., Nelson, W.G., Huang, J. W., Woodcock-Mitchel, J. and Sun, T.T. (1982). *Cell*. 30:361-372.
3. Asch, B.B. and Asch, H.L. (1986). *Cancer Research*. 46:1255.
4. Rodriguez, M.M., Krachmer, J.H. and Sun, T.T. (1986). *Trans Am. Opthalmo. Soc*. 84:146.
5. Clausen, H. Vedtofte, P. Moe, D., Dabelsteen, E. Sun, T.T. and Dale, B. (1986). *J. Invest. Dermatol*. 86:249.
6. Klein-Szanto, A.J., Boysen, M. and Reith, A. (1987) *Arch. Pathol. Lab. Med*. 111:1057.
7. Reibel, J., Scholdt, M. and Dabelsteen, E. (1985) *Acta Pathol.Microbiol. Immunol. Scand*. 93; 159.



Western Blot Analysis: Representative lot data. A431 lysate was resolved by electrophoresis, transferred to PVDF membrane and probed with anti-CYTOKERATIN AE1/AE3 (1:500 dilution). Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates protein CYTOKERATIN AE1/AE3 (~40-70 kDa).

APPLICATION LEGEND: **WB** Western Blotting **ELISA** Enzyme-linked Immunosorbent Assay **IP** Immunoprecipitation **IC** Immunocytochemistry **IF** Immunofluorescence **IH** Immunohistochemistry (Tissue)

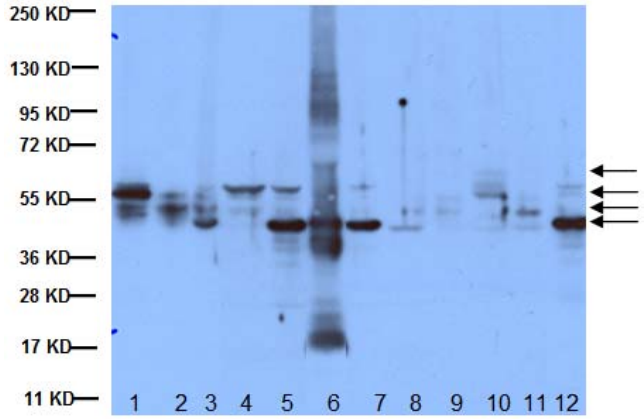
SPECIES LEGEND: **H** Human **M** Mouse **R** Rat **Rb** Rabbit **Mk** Monkey **B** Bovine **Ch** Chicken

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

Submit your published journal article, and earn credit toward future Millipore purchases. Visit www.millipore.com/publicationrewards to learn more!

Rev.C/2014-02-20/MAB3412CA/RB

Additional Research Applications



Western Blot Analysis:
Representative image from a previous lot.
A431 (Lane1), C2C12 lysate (Lane2), HEK293 lysate (Lane 3), Hela lysate (Lane 4), HepG2 lysate (Lane 5), Human Placenta lysate (Lane 6), Huvec lysate (Lane 7), Jurkat lysate (Lane 8), L6 lysate (Lane 9), Mouse brain lysate (Lane 10), NIH/3T3 lysate (Lane 11), and PC3 lysate (Lane 12) were resolved by electrophoresis, transferred to PVDF membrane and probed with anti-CYTOKERATIN AE1/AE3(1:500 dilution of a previous lot).
Proteins were visualized using a goat anti-mouse secondary antibody conjugated to HRP and a chemiluminescence detection system.

Arrow indicates protein CYTOKERATIN AE1/AE3 (~40-70 kDa).

ELISA: A previous lot of this antibody was used for ELISA (Woodcock-Mitchel & Sun, 1982).

Immunohistochemistry(paraffin): 0.5-2 µg/mL of a previous lot was used on staining of unfixed frozen or formalin -fixed, paraffin-embedded tissue section (Woodcock-Mitchel & Sun, 1982; Tseng et al., 1982; Asch * Asch, 1986; Rodriguez et al., 1986; Clausen et al., 1986; Klein-Szanto et al., 1987; Reibel et al., 1985). Trypsin or pepsin digestion is required for proper staining on paraffin embedded tissues. {Trypsin 1 mg/mL 10 minutes, 37°C, or pepsin 1 mg/mL 5 minutes 37°C}. High temperature with citrate antigen retrieval can also be used.

Optimal dilutions must be determined by end user.

PROTOCOL

Western Blotting

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% milk 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 AE1/AE3 diluted in freshly prepared 5% milk 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 donkey anti-mouse HRP conjugate IgG, Catalog # AP192P 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.

Immunohistochemstry

Proteolytic treatment enhances specific staining of formalin-fixed paraffin-embedded tissue sections and allows higher dilutions of the antibody to be used (Pinkus et al., 1985).

Preparations of Slides:

1. Prepare 5 µm sections from tissue fixed with 10% buffered formalin and embedded in paraffin.
2. De-wax, clear with xylene, and hydrate tissue sections through a graded series of alcohol solutions.
3. If using a peroxidase secondary antibody, block endogenous peroxidase by incubating slides in 2% H_2O_2 in methanol for 15-20 min at room temperature.
4. Wash slides with running demonized water for 2 min.

Slides may be stored in 0.05 M Tris-buffered saline (TBS), pH 7.6, for up to 24 h at +4°C.

Trypsin Digestion

1. Prepare 50 mL of 0.1% $CaCl_2$ (w/v) in TBS $CaCl_2$-TBS).
2. Prepare 0.1% trypsin solution (w/v) in $CaCl_2$-TBS.
3. Warm the 0.1% trypsin solution for 20 min in a +37°C water bath. Trypsin must be used within an hour after preparation.
4. Incubate the slides for 30 min in the 0.1% trypsin solution at +37°C.
5. Wash slides with running deionized water for 3 min.

Tissue Staining with anti-keratin AE1/AE3 monoclonal antibody

1. Block non-specific binding by incubating sections in 2.5% normal animal serum (v/v) diluted in TBS that contains 0.1% bovine serum albumin (TBS-BSA). The animal serum should be from the same species as the secondary antibody.
2. Drain off excess serum (without washing) and replace with 100-300 µL of anti-keratin AE1/AE3 monoclonal antibody diluted in TBS-BSA. Incubate at room temperature for 60 minutes in a humid chamber. **Note**: The optimal concentration of anti-keratin AE1/AE3 depends on the secondary antibody detection system. When AE1/AE3 binding is detected with a biotinylated secondary antibody-avidin-biotinylated peroxidase system according to S.M. Hsu et al. (Hsu, et al., 1981), a 1-10 µg/mL anti-keratin AE1/AE3 solution can be used. For optimal results, both the primary and secondary antibody reagents should be titrated (Taylor, 1978).
3. Wash with TBS, changing the solution 3 times over a 10-minute period.
4. Detect with a standard secondary antibody detection system (Falini & Taylor, 1983; Harlow & Lane, 1988).

RELATED PRODUCTS (specific)

cat #	description
MAB3224	Anti-Basal Cell Cytokeratin
CBL234F	Anti-Cytokeratin Pan, Clone C-11, FITC Conjugated
MAB3406-200UG	Anti-Cytokeratin Pan, clone Lu5
MAB3406-40UG	Anti-Cytokeratin Pan, clone Lu5
CBL234	Anti-Pan-Cytokeratin, clone C11
IHCR2025-6	IHC Select® Anti-Cytokeratin AE1/AE3 (Pan cytokeratins), prediluted, clone AE1/AE3
IHCR2029-6	IHC Select® Anti-Cytokeratin High MW, prediluted, clone 34betaE12
IHCR2026-6	IHC Select® Anti-Cytokeratin Type I, prediluted, clone AE1
IHCR2027-6	IHC Select® Anti-Cytokeratin Type II, prediluted, clone 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
AP124P	Goat anti-Mouse IgG, Peroxidase Conjugated, H+L

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
WBKLS0100	Immobilon Western Chemilum HRP Substrate 100 mL
17-373	Spray & Glow™ ECL WB Detection System 1 ea
2060	Re-Blot Western Blot Recycling Kit
2500	Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	Blot Quick Blocker Membrane Blocking Agent 175G