

Monoclonal Antibody to Cytokeratin (Hair Cortex) - Purified

Catalog No.: BM4538

Quantity: 0.1 ml

Concentration: 1 mg/ml

Background: The keratins are intermediate filament proteins responsible for the structural integrity of epithelial cells and are subdivided into cytokeratins and hair keratins. Most of the type I cytokeratins consist of acidic proteins which are arranged in pairs of heterotypic keratin chains and are clustered in a region on chromosome 17q21.2.

Host / Isotype: Mouse / IgG

Clone: AE13

Immunogen: Human hair keratins.

Format: **State:** Liquid purified IgG fraction
Purification: Protein G Chromatography
Buffer System: PBS, pH 7.2
Preservatives: 0.09% Sodium Azide

Applications: **Immunoblotting:** 1/1000-1/3000. Detects a band of approximately 44 kDa.
Flow Cytometry: 1/20-1/50.
Immunohistochemistry on Frozen Sections (Ref.1-7).
Immunohistochemistry on Paraffin Sections.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Molecular Weight: 44-46 kDa (Predicted)

Specificity: This antibody recognises Acidic 44-46 kDa hair cortex keratins.
AE13 is an excellent marker for hair and nail differentiation.
Species: Human and Mouse.
Other species not tested.

Storage: Store undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

General Readings: 1. Chen D, Jarrell A, Guo C, Lang R, Atit R. Dermal β -catenin activity in response to epidermal Wnt ligands is required for fibroblast proliferation and hair follicle initiation. *Development*. 2012 Apr;139(8):1522-33. doi: 10.1242/dev.076463. PubMed PMID: 22434869.
2. Larouche D, Cuffley K, Paquet C, Germain L. Tissue-engineered skin preserving the potential of epithelial cells to differentiate into hair after grafting. *Tissue Eng Part A*. 2011 Mar;17(5-6):819-30. doi: 10.1089/ten.TEA.2010.0403. Epub 2010 Dec 14. PubMed PMID: 20973750.

3. Youssef KK, Van Keymeulen A, Lapouge G, Beck B, Michaux C, Achouri Y, et al. Identification of the cell lineage at the origin of basal cell carcinoma. *Nat Cell Biol.* 2010 Mar;12(3):299-305. doi: 10.1038/ncb2031. Epub 2010 Feb 14. PubMed PMID: 20154679.
4. Van Keymeulen A, Mascre G, Youseff KK, Harel I, Michaux C, De Geest N, et al. Epidermal progenitors give rise to Merkel cells during embryonic development and adult homeostasis. *J Cell Biol.* 2009 Oct 5;187(1):91-100. doi: 10.1083/jcb.200907080. Epub 2009 Sep 28. PubMed PMID: 19786578.
5. List K, Szabo R, Molinolo A, Nielsen BS, Bugge TH. Delineation of matriptase protein expression by enzymatic gene trapping suggests diverging roles in barrier function, hair formation, and squamous cell carcinogenesis. *Am J Pathol.* 2006 May;168(5):1513-25. PubMed PMID: 16651618.
6. Kajikawa M, Baba T, Tomaru U, Watanabe Y, Koganei S, Tsuji-Kawahara S, et al. MHC class I-like MILL molecules are beta2-microglobulin-associated, GPI-anchored glycoproteins that do not require TAP for cell surface expression. *J Immunol.* 2006 Sep 1;177(5):3108-15. PubMed PMID: 16920948.
7. Sun Y, Strizzi L, Raafat A, Hirota M, Bianco C, Feigenbaum L, et al. Overexpression of human Cripto-1 in transgenic mice delays mammary gland development and differentiation and induces mammary tumorigenesis. *Am J Pathol.* 2005 Aug;167(2):585-97. PubMed PMID: 16049342.
8. Suzuki K, Yamaguchi Y, Villacorte M, Mihara K, Akiyama M, Shimizu H, et al. Embryonic hair follicle fate change by augmented beta-catenin through Shh and Bmp signaling. *Development.* 2009 Feb;136(3):367-72. doi: 10.1242/dev.021295. PubMed PMID: 19141668.
9. Lynch MH, O'Guin WM, Hardy C, Mak L, Sun TT. Acidic and basic hair/nail ("hard") keratins: their colocalization in upper cortical and cuticle cells of the human hair follicle and their relationship to "soft" keratins. *J Cell Biol.* 1986 Dec;103(6 Pt 2):2593-606. PubMed PMID: 2432071.
10. Dhouailly D, Xu C, Manabe M, Schermer A, Sun TT. Expression of hair-related keratins in a soft epithelium: subpopulations of human and mouse dorsal tongue keratinocytes express keratin markers for hair-, skin- and esophageal-types of differentiation. *Exp Cell Res.* 1989 Mar;181(1):141-58. PubMed PMID: 2465162.

Protocols:**Immunofluorescence Protocol - Formaldehyde Fixation**

Collect cells from T.c.unit and remove media from petri dish using suction.

Wash with 1x PBS and remove.

Incubate cells in pre-warm (37°C) Para-Formaldehyde for 12 minutes at room temperature on an orbital shaker.

Remove PFA and incubate in 0.5% Triton X-100 in 1x PBS for 5 minutes at room temperature.

Prepare blocking reagent, this is also the antibody diluent.

Wash cells 2x with 1x PBS at room temperature, for 4 minutes/wash on an orbital shaker.

Block with 1% NCS and 1x PBS for 30 minutes at room temperature.

Prepare primary antibodies (50µl/coverslip) and moist staining chambers.

Wash cells 2x with 1x PBS at room temperature and air dry briefly.

Incubate with primary antibody for 1 hr at room temperature in the dark in staining chambers. During this time prepare the secondary antibody.

Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)

Incubate with secondary antibody for 1 hour at room temperature in the dark in staining chambers.

Wash cells 5x with 1x PBS.

Mount in Dapi.

Solutions (prepare fresh the same day of staining):

1x Phosphate buffered saline.

Blocking reagent: 1% NCS in 1x PBS (use fresh 10x PBS).

Fixation Solution: 3.5% Para formaldehyde.

1.75g PFA in 20 ml d.H₂O plus 5 drops 1M NaOH. Stir on a hot plate at 50-60°C until dissolved. Add 4 drops IN HCl and check pH indicator strip. PH should be 7.4. Complete volume with d.H₂O to 25ml and add 25ml 2xPBS. Check pH before adding to cover slips.

Immunofluorescence protocol - Methanol/Acetone Fixation

Collect cells from T.C.unit and remove media from petri dish using suction.

Wash with 1x PBS and remove.

Fix cells with cold methanol: acetone 1:1 for 10 minutes on ice.

Prepare blocking reagent, this is also the diluent for the antibodies.

Remove fixative and wash cells 3x with 1x PBS at RT, for 4 minutes/wash on orbital shaker.

Block with 1% NCS and 1x PBS for 30 minutes at RT.

Prepare primary antibodies (50µl/coverslip) and moist staining chambers.

Wash cells 2x with 1 x PBS at RT and air dry for approximately 7 minutes.

Incubate with primary antibody for 1 hr at RT in the dark in staining chambers. During this time prepare secondary antibody.

Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)

Incubate with secondary antibody for 1 hr at R T in the dark in staining chambers.

Wash cells 5x with 1x PBS.

Mount in Dapi.

Solutions (prepare fresh the same day of staining):

1x Phosphate buffered saline.

Blocking reagent: 1% NCS in 1x PBS (use fresh 10x PBS).

Fixation solution: methanol:acetone 1: 1 ice cold.

Western Blotting Protocol

Transfer gel to PDVF or nitrocellulose membrane

Place membrane in plastic tray in blocking buffer for one hour with agitation

Rinse in wash buffer

Incubate in wash buffer plus primary antibody for one hour

Wash 6 X 5 minutes with wash buffer

Incubate in wash buffer plus secondary antibody for one hour

Wash 6X 5 minutes with wash buffer

Detect (e.g. ECL, Amersham according to manufacturers instructions)

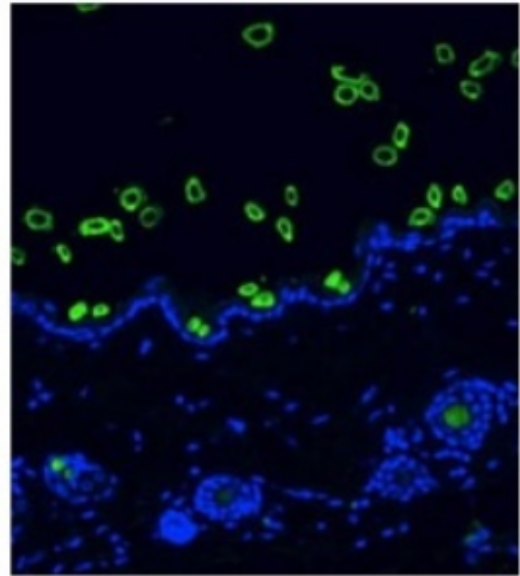
Wash buffer: PBS + 0.1% Tween 20

Blocking buffer: Wash buffer + 5% dried milk powder

The concentration of antibodies used depends on each antibody, the amount of antigen and the detection method used. Generally, dilution is in the range of a few hundred times dilution to a few thousand times dilution, but usually has to be determined empirically.

Pictures:

AE13 Antibody at 1/100 staining mouse skin tissue sections by Immunohistochemistry (paraffin). The tissue was paraformaldehyde fixed and blocked with BSA. A heat mediated antigen retrieval step was performed. The tissue was incubated with the antibody for 16 hours and then an Alexa-Fluor488 conjugated goat anti-mouse antibody was used as the secondary. The image shows hair cortex cytokeatin staining in green with DAPI nuclei counterstain in blue.



AE13 Antibody staining hair cortex Cytokeratin in adult mouse nail by Immunohistochemistry (paraffin embedded sections). Tissue was fixed with paraformaldehyde and blocked with 10% serum for 1 hour at 20°C followed by incubation with the primary antibody, at a 1/200 dilution, for 12 hours at 4°C. A Biotinconjugated goat anti-mouse monoclonal was used as secondary antibody at a 1/400 dilution.

