OriGene Technologies Inc.

9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

OriGene EU

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Monoclonal Antibody to Epidermal Cytokeratin - Purified

Catalog No.: BM4539

Quantity: 0.1 ml

Background: This gene encodes a member of the type I (acidic) cytokeratin family, which belongs to the superfamily of intermediate filament (IF) proteins. Keratins are heteropolymeric structural proteins which form the intermediate filament. These filaments, along with actin microfilaments and microtubules, compose the cytoskeleton of epithelial cells. Mutations in this gene are associated with epidermolytic hyperkeratosis. This gene is located within a cluster of keratin family members on chromosome 17q21.

Host / Isotype: Mouse / IgG

Clone: AE20

Immunogen: Human epidermal keratins.

Format: **State:** Liquid purified Ig fraction.
Purification: Protein G chromatography.
Buffer System: PBS with 0.09% sodium azide as preservative.

Applications: Suitable for Immunblotting (1/1000-1/3000 for Western blot) and Immunohistochemical staining of Paraffin Sections (10 µg/ml).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Human keratin K10-1 (56.5 Kda).

Storage: Store the antibody 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. Tseng SC, Jarvinen MJ, Nelson WG, Huang JW, Woodcock-Mitchell J, Sun TT. Correlation of specific keratins with different types of epithelial differentiation: monoclonal antibody studies. Cell. 1982 Sep;30(2):361-72. PubMed PMID: 6183000.
2. Woodcock-Mitchell J, Eichner R, Nelson WG, Sun TT. Immunolocalization of keratin polypeptides in human epidermis using monoclonal antibodies. J Cell Biol. 1982 Nov;95(2 Pt 1):580-8. PubMed PMID: 6183275.
3. Cooper D, Schermer A, Sun TT. Classification of human epithelia and their neoplasms using monoclonal antibodies to keratins: strategies, applications, and limitations. Lab Invest. 1985 Mar;52(3):243-56. PubMed PMID: 2579289.
4. Loomis CA, Kolega J, Manabe M, Sun TT. Characterization of a keratinocyte-specific extracellular epitope of desmoglein. Implications for desmoglein heterogeneity and function. J Biol Chem. 1992 Aug 15;267(23):16676-84. PubMed PMID: 1379602.

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-IOO 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 lx 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 l0x PBS).
Fixation solution: 3.5% Para formaldehyde.
1.75g PFA in 20 ml d.H20 plus 5 drops 1M NaOH. Stir on a hot plate at 50-60°C until dissolved. Add 4 drops IN HCI and check pH indicator strip. PH should be 7.4. Complete volume with d.H20 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 Ix PBS at RT, for 4 minutes/wash on orbital shaker.
Block with 1% NCS and Ix 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.

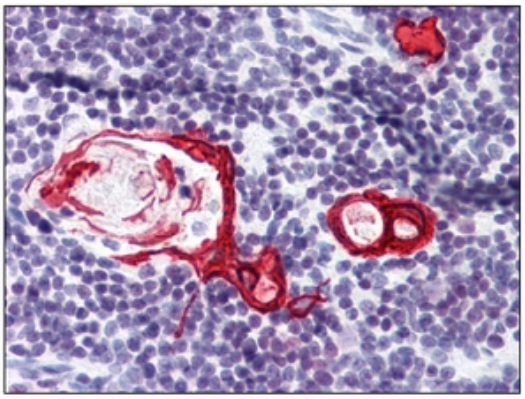
Western Blotting Protocol

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:

BM4539 KRT10 antibody staining of Formalin-Fixed, Paraffin-Embedded Human Thymus at 10 µg/ml followed by biotinylated anti-mouse IgG secondary antibody, alkaline phosphatase-streptavidin and chromogen.



BM4539 KRT10 antibody staining of Formalin-Fixed, Paraffin-Embedded Human Skin at 10 µg/ml followed by biotinylated anti-mouse IgG secondary antibody, alkaline phosphatase-streptavidin and chromogen.