

Anti-Cytokeratin 14 Antibody [SC65-06] - BSA and Azide free

HA750216



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human, Mouse, Rat

Applications: WB, IF-Cell, IF-Tissue, IHC-P, FC

Molecular Wt: Predicted band size: 51 kDa

Clone number: SC65-06

Description: This gene encodes a member of the keratin family, the most diverse group of intermediate filaments. This gene product, a type I keratin, is usually found as a heterotetramer with two keratin 5 molecules, a type II keratin. Together they form the cytoskeleton of epithelial cells. Mutations in the genes for these keratins are associated with epidermolysis bullosa simplex. The nonhelical tail domain is involved in promoting KRT5-KRT14 filaments to self-organize into large bundles and enhances the mechanical properties involved in resilience of keratin intermediate filaments in vitro. Expressed in the corneal epithelium (at protein level). Detected in the basal layer, lowered within the more apically located layers specifically in the stratum spinosum, stratum granulosum but is not detected in stratum corneum. Strongly expressed in the outer root sheath of anagen follicles but not in the germinative matrix, inner root sheath or hair. A form of epidermolysis bullosa simplex, a group of skin fragility disorders characterized by skin blistering due to cleavage within the basal layer of keratinocytes, and erosions caused by minor mechanical trauma. There is a broad spectrum of clinical severity ranging from minor blistering on the feet, to subtypes with extracutaneous involvement and a lethal outcome. EBS1A is an autosomal dominant form characterized by generalized intraepidermal skin blistering that begins and is very prominent at birth. EBS1A may be life-threatening in the first year of life. Tendency to blistering diminishes in adolescence.

Immunogen: Recombinant protein within Mouse Cytokeratin 14 aa 250-484 / 484.

Positive control: A431 cell lysate, mouse skin tissue lysate, B16F1, SW480, HepG2, human cervical carcinoma tissue, human skin tissue, rat skin tissue, mouse skin tissue, mouse prostate tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P02533 Human | Q61781 Mouse | Q6IFV1 Rat

Recommended Dilutions:

WB 1:2,000

IF-Cell 1:100-1:500

IF-Tissue 1:100-1:500

IHC-P 1:100-1:1,500

FC 1:1,000

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4°C after thawing. Aliquot store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

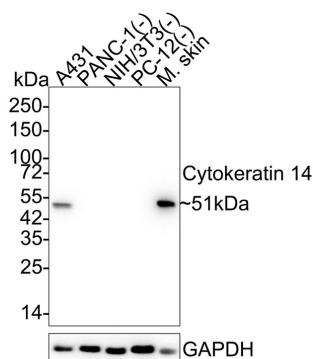
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Images

Fig1: Western blot analysis of Cytokeratin 14 on different lysates with Rabbit anti-Cytokeratin 14 antibody (HA750216) at 1/2,000 dilution.



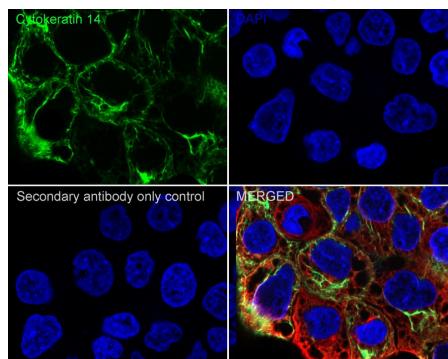
Lane 1: A431 cell lysate (15 µg/Lane)
 Lane 2: PANC-1 cell lysate (negative) (15 µg/Lane)
 Lane 3: NIH/3T3 cell lysate (negative) (15 µg/Lane)
 Lane 4: PC-12 cell lysate (negative) (15 µg/Lane)
 Lane 5: Mouse skin tissue lysate (20 µg/Lane)

Predicted band size: 51 kDa
 Observed band size: 51 kDa

Exposure time: 24 seconds;
 4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750216) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Immunocytochemistry analysis of A431 cells labeling Cytokeratin 14 with Rabbit anti-Cytokeratin 14 antibody (HA750216) at 1/500 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Cytokeratin 14 antibody (HA750216) at 1/500 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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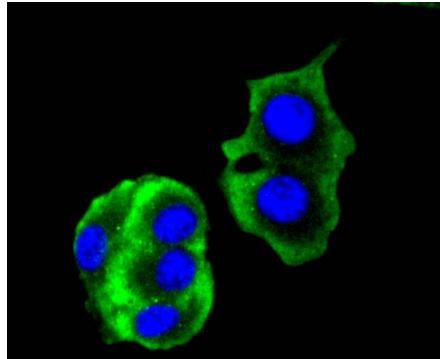


Fig3: ICC staining of Cytokeratin 14 in B16F1 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750216, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

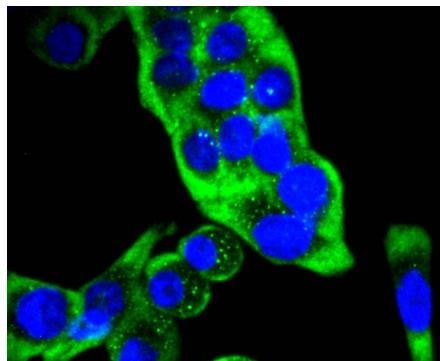


Fig4: ICC staining of Cytokeratin 14 in SW480 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750216, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

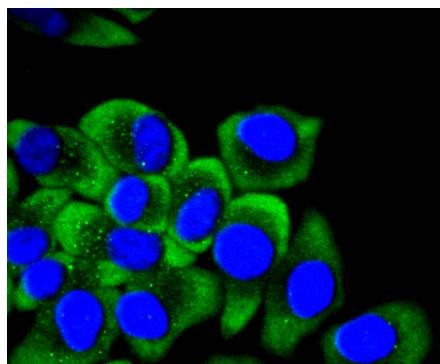


Fig5: ICC staining of Cytokeratin 14 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (HA750216, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).

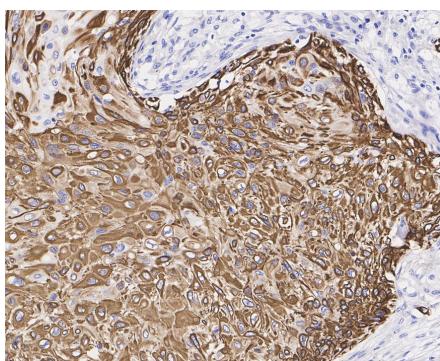


Fig6: Immunohistochemical analysis of paraffin-embedded human cervical carcinoma tissue with Rabbit anti-Cytokeratin 14 antibody (HA750216) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750216) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

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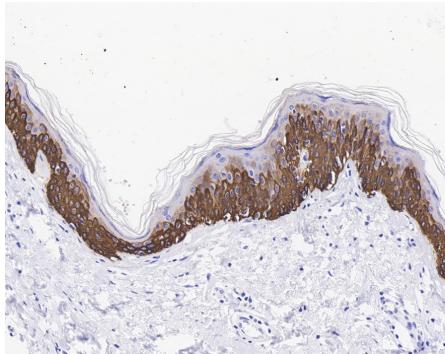


Fig7: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-Cytokeratin 14 antibody (HA750216) at 1/1,500 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750216) at 1/1,500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

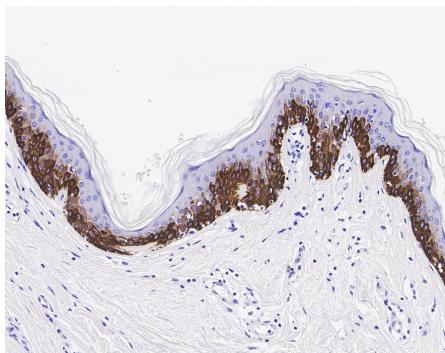


Fig8: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-Cytokeratin 14 antibody (HA750216) at 1/1,500 dilution.

The section was not undergone antigen retrieval. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750216) at 1/1,500 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

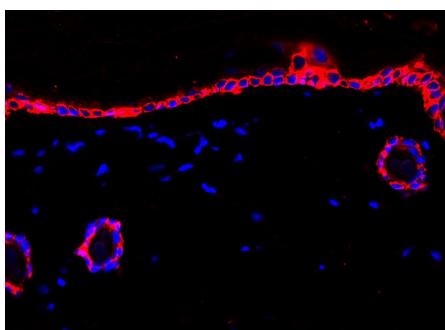


Fig9: Immunofluorescence analysis of paraffin-embedded rat skin tissue labeling Cytokeratin 14 (HA750216).

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies Cytokeratin 14 (ET1610-42, red) at 1/500 dilution at +4°C overnight, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) was used as the secondary antibodies at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

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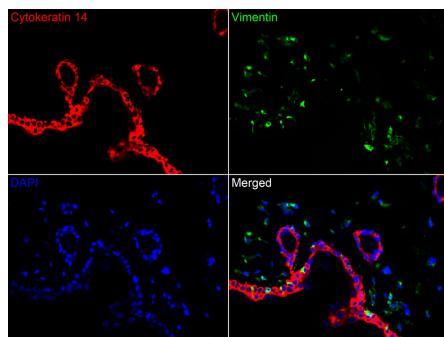
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Fig10: Immunofluorescence analysis of paraffin-embedded rat skin tissue labeling Cytokeratin 14 (HA750216) and Vimentin (EM0401).



The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies Cytokeratin 14 (ET1610-42, red) at 1/400 dilution and Vimentin (EM0401, green) at 1/400 dilution at +4°C overnight, washed with PBS.

Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) and Goat Anti-Mouse IgG H&L (iFluor™ 488, HA1125) were used as the secondary antibodies at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

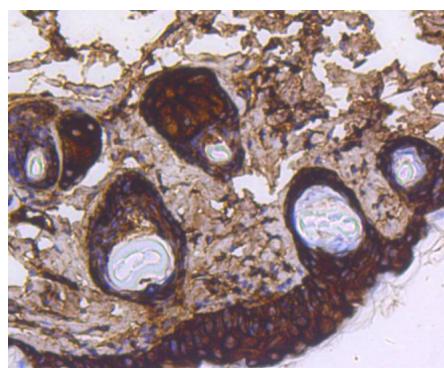


Fig11: Immunohistochemical analysis of paraffin-embedded mouse skin tissue using anti-Cytokeratin 14 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750216, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

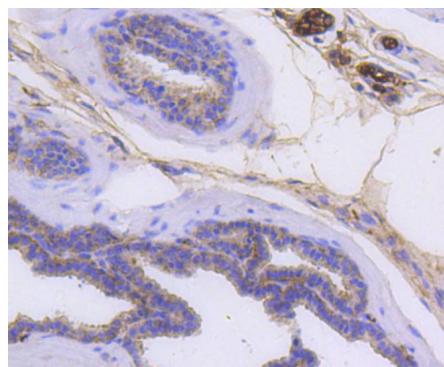
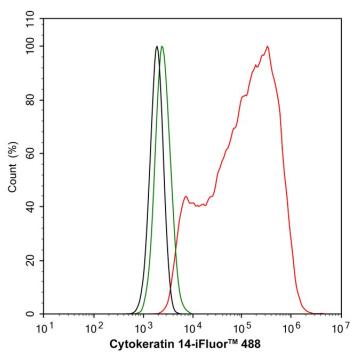


Fig12: Immunohistochemical analysis of paraffin-embedded mouse prostate tissue using anti-Cytokeratin 14 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750216, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Fig13: Flow cytometric analysis of A431 cells labeling Cytokeratin 14.



Cells were fixed and permeabilized. Then stained with the primary antibody (HA750216, 1µg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Pastar I et al. Interactions of methicillin resistant *Staphylococcus aureus* USA300 and *Pseudomonas aeruginosa* in polymicrobial wound infection. *PLoS One* 8:e56846 (2013).
2. DeWard AD et al. Cellular heterogeneity in the mouse esophagus implicates the presence of a nonquiescent epithelial stem cell population. *Cell Rep* 9:701-11 (2014).

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Applications: WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF=Cell=Immunofluorescence (Cell) IF=Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation