Anti-Cytokeratin 14 Antibody [SC65-06]

ET1610-42

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 51 kDa
Clone number:	SC65-06

Description: Cytokeratins comprise a diverse group of intermediate filament proteins (IFPs) that are expressed in pairs in both keratinized and non-keratinized epithelial tissue, where they constitute up to 85% of mature keratinocytes in the vertebrate epidermis. Cytokeratins play a critical role in differentiation and tissue specialization and function to maintain the overall structural integrity of epithelial cells. The a-helical coiled-coil dimers associate laterally end-to-end to form 10 nm diameter filaments. Cytokeratins are useful markers of tissue differentiation and, in addition, they aid in the characterization of malignant tumors. In Bowen's disease, the characteristic malignancy of the epidermis exhibits distinct expression patterns of Cytokeratin 14. The gene encoding human Cytokeratin 14 maps to chromosome 17q12-21. Mutations in this gene lead to epidermolysis bullosa simplex, an inheritied skin disorder characterized by skin blistering due to basal keratinocyte fragility.

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

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

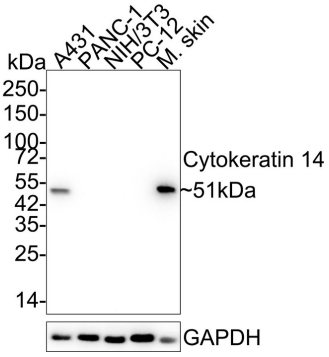

Fig1: Western blot analysis of Cytokeratin 14 on different lysates with Rabbit anti-Cytokeratin 14 antibody (ET1610-42) at 1/2,000 dilution.

Lane 1: A431 cell lysate (15 µg/Lane)
Lane 2: PANC-1 cell lysate (15 µg/Lane) (negative)
Lane 3: NIH/3T3 cell lysate (15 µg/Lane) (negative)
Lane 4: PC-12 cell lysate (15 µg/Lane) (negative)
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 (ET1610-42) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

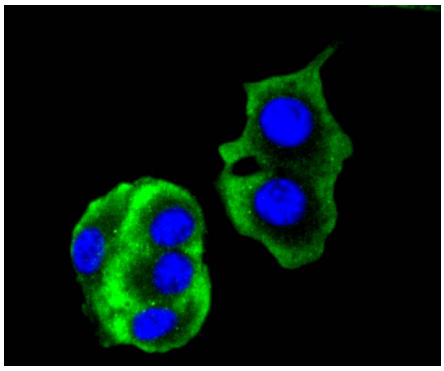

Fig2: ICC staining of Cytokeratin 14 in B16F1 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1610-42, 1/50) for 1 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).

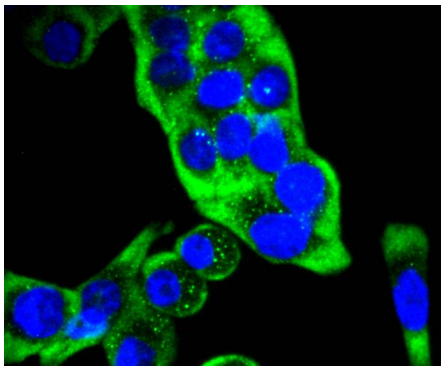

Fig3: ICC staining of Cytokeratin 14 in SW480 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1610-42, 1/50) for 1 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).

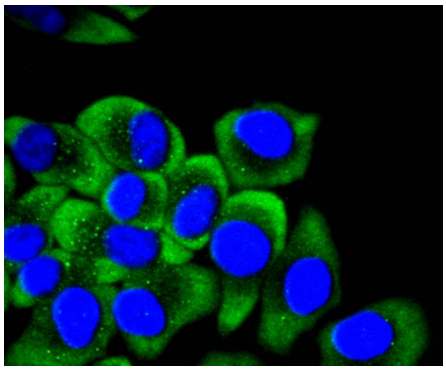

Fig4: ICC staining of Cytokeratin 14 in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1610-42, 1/50) for 1 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).

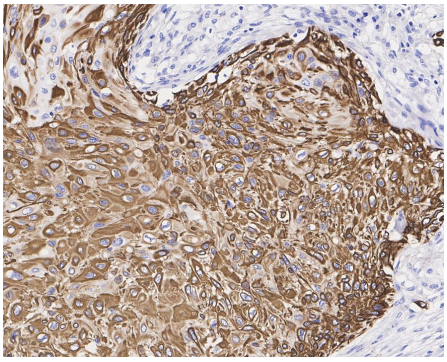

Fig5: Immunohistochemical analysis of paraffin-embedded human cervical carcinoma tissue with Rabbit anti-Cytokeratin 14 antibody (ET1610-42) at 1/1,000 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1610-42) 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.

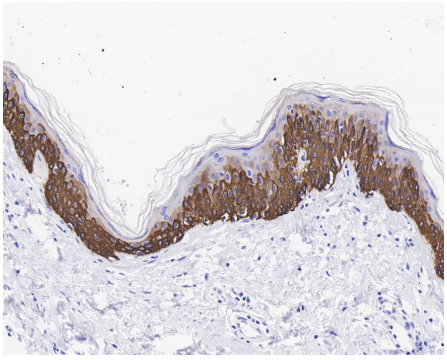

Fig6: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-Cytokeratin 14 antibody (ET1610-42) at 1/1,500 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1610-42) 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.

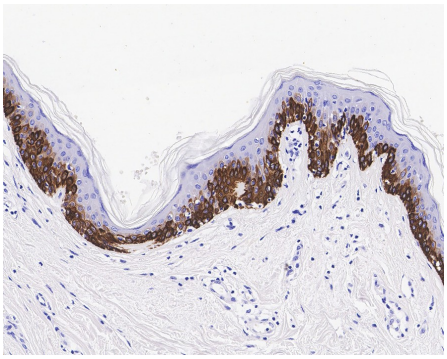

Fig7: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-Cytokeratin 14 antibody (ET1610-42) at 1/1,500 dilution.

The section was not undergone antigen retrieval. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-42) 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.

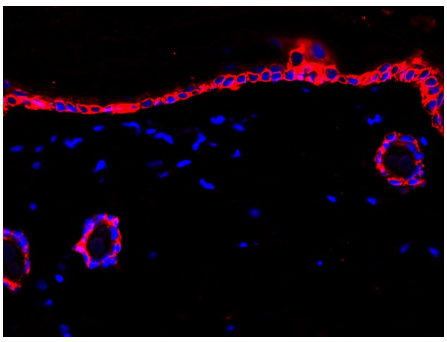

Fig8: Immunofluorescence analysis of paraffin-embedded rat skin tissue labeling Cytokeratin 14 (ET1610-42).

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℃ 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).

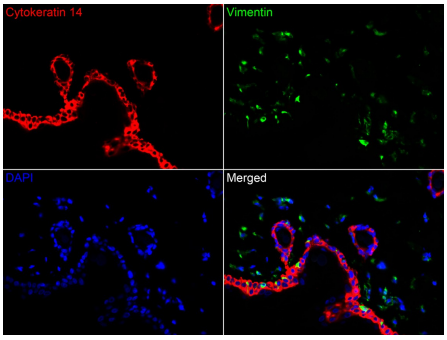

Fig9: Immunofluorescence analysis of paraffin-embedded rat skin tissue labeling Cytokeratin 14 (ET1610-42) and Vimentin (EM0401).

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℃ 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).

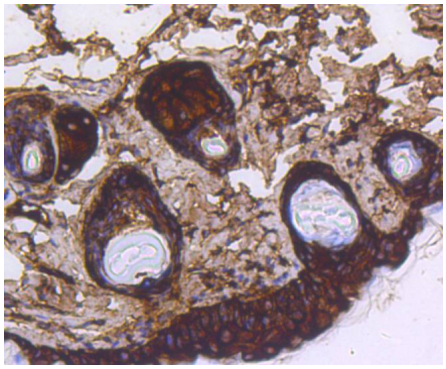

Fig10: Immunohistochemical analysis of paraffin-embedded mouse skin tissue using anti-Cytokeratin 14 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-42, 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.

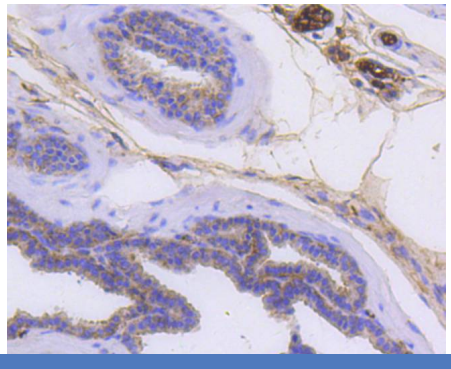

Fig11: Immunohistochemical analysis of paraffin-embedded mouse prostate tissue using anti-Cytokeratin 14 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-42, 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

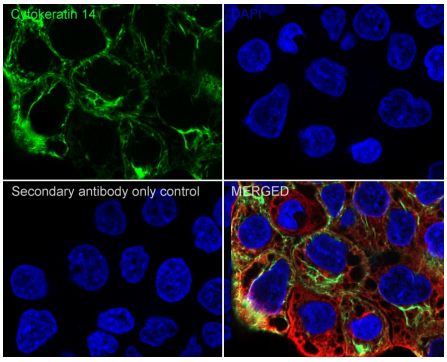

Fig12: Immunocytochemistry analysis of A431 cells labeling Cytokeratin 14 with Rabbit anti-Cytokeratin 14 antibody (ET1610-42) at 1/500 dilution.

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 (ET1610-42) at 1/500 dilution in 1% BSA in PBST overnight at 4 ℃. 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℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

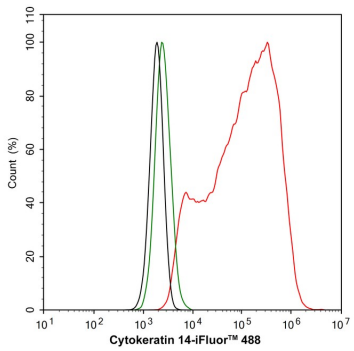

Fig13: Flow cytometric analysis of A431 cells labeling Cytokeratin 14.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1610-42, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ 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℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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).