Anti-Cytokeratin 5 Antibody [SC62-04]

ET1610-43

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue, IF-Cell, FC
Molecular Wt:	Predicted band size: 62 kDa
Clone number:	SC62-04

Description: Cytokeratin 5 is commonly found in the cells on the outermost layer of skin in humans and animals. It is encoded by the KRT5 gene and pairs with the type I keratin K14. Cytokeratin 5 has become an important biomarker for different types of cancer, including mesothelioma, breast cancer and lung cancer. It helps differentiate squamous carcinomas from adenocarcinomas. Pathologists use cytokeratin 5 to distinguish mesothelioma from adenocarcinoma, the most common type of lung cancer. Cytokeratin 5/6 cannot identify mesothelioma on its own. Cytokeratin 5/6 is a positive marker for malignant pleural mesothelioma, found in more than three-fourths of cases. It is also found in certain types of lung cancers and breast cancers. Pathologists use cytokeratin 5/6 to stain cancer tissue samples. Cytokeratin 5/6 immunoreactivity is rarely seen in adenocarcinomas of the lung. If a tumor sample shows strong expression of cytokeratin 5/6, it gives pathologists a hint the tumor is malignant mesothelioma rather than a metastatic adenocarcinoma. However, this marker is not effective for all cell types of mesothelioma. Cytokeratin 5/6 staining is usually weak or negative for sarcomatoid mesothelioma, the least common and hardest-to-treat cell type of the asbestos-related cancer. The marker is also not effective in telling the difference between pleural mesothelioma and squamous cell carcinomas. About 25 to 30 percent of all lung cancers are squamous cell carcinomas.

Immunogen: Synthetic peptide within human Cytokeratin 5 aa 70-110.

Positive control: A431, A431 cell lysate, MDA-MB-468 cell lysate, MCF7 cell lysate, mouse skin tissue lysate, rat skin tissue lysate, human skin tissue, human tonsil tissue, human lung squamous cell carcinoma tissue, human breast carcinoma tissue.

Subcellular location: Cytoplasm.

Database links: SwissProt: P13647 Human | Q922U2 Mouse | Q6P6Q2 Rat

Recommended Dilutions:

WB	1:5,000
IHC-P	1:50-1:1,500
IF-Tissue	1:200
IF-Cell	1:500
FC	1:1,000

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

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

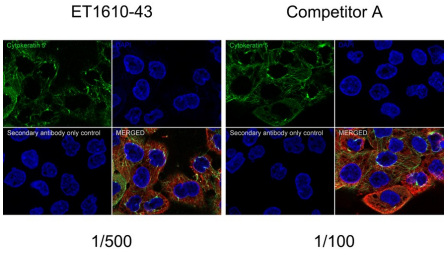

Fig1: Immunocytochemistry analysis of A431 cells labeling Cytokeratin 5 with Rabbit anti-Cytokeratin 5 antibody (ET1610-43) at 1/500 dilution and competitor's antibody at 1/100 dilution.

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 5 antibody (ET1610-43) at 1/500 dilution and competitor's antibody at 1/100 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.

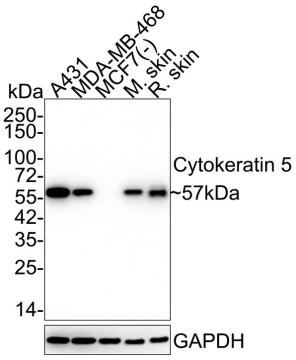

Fig2: Western blot analysis of Cytokeratin 5 on different lysates with Rabbit anti-Cytokeratin 5 antibody (ET1610-43) at 1/5,000 dilution.

Lane 1: A431 cell lysate
Lane 2: MDA-MB-468 cell lysate
Lane 3: MCF7 cell lysate (negative)
Lane 4: Mouse skin tissue lysate
Lane 5: Rat skin tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 62 kDa
Observed band size: 57 kDa

Exposure time: 2 minutes 6 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 (ET1610-43) at 1/5,000 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.

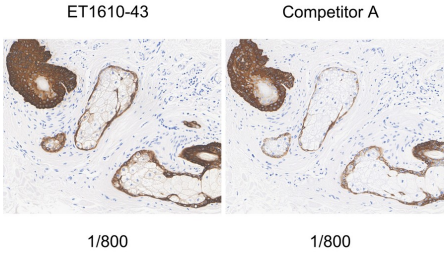

Fig3: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-Cytokeratin 5 antibody (ET1610-43) at 1/800 dilution and competitor's antibody at 1/800 dilution.

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 (ET1610-43) at 1/800 dilution and competitor's antibody at 1/800 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.

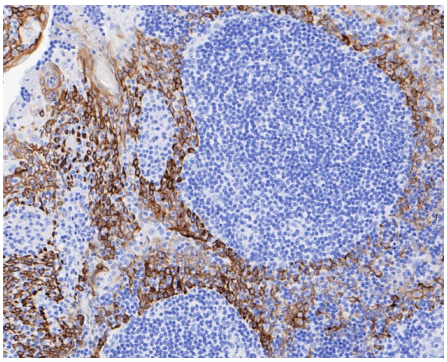

Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Cytokeratin 5 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-43, 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.

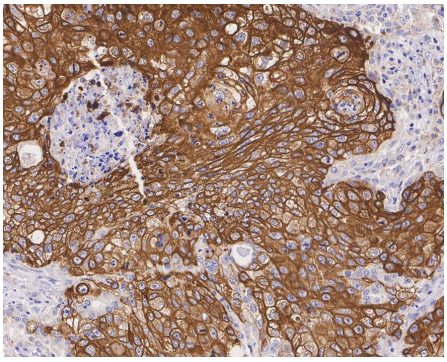

Fig5: Immunohistochemical analysis of paraffin-embedded human lung squamous cell carcinoma tissue with Rabbit anti-Cytokeratin 5 antibody (ET1610-43) at 1/1,500 dilution.

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 (ET1610-43) 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.

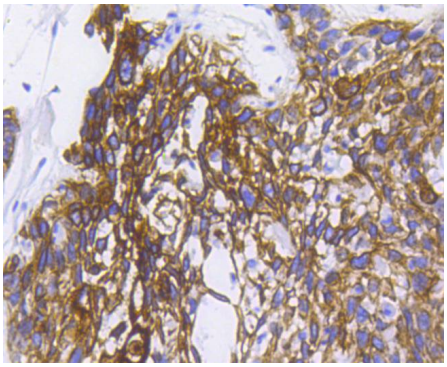

Fig6: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Cytokeratin 5 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-43, 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.

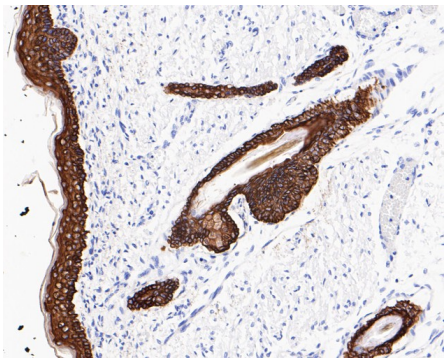

Fig7: Immunohistochemical analysis of paraffin-embedded human skin tissue using anti-Cytokeratin 5 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1610-43, 1/200) 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.

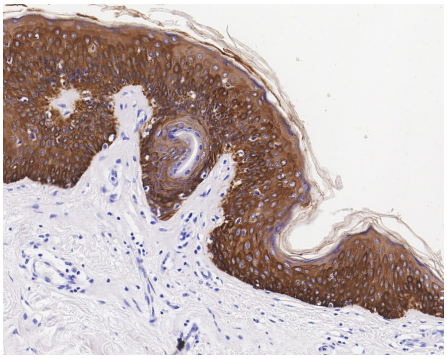

Fig8: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-Cytokeratin 5 antibody (ET1610-43) at 1/1,500 dilution.

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 (ET1610-43) 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.

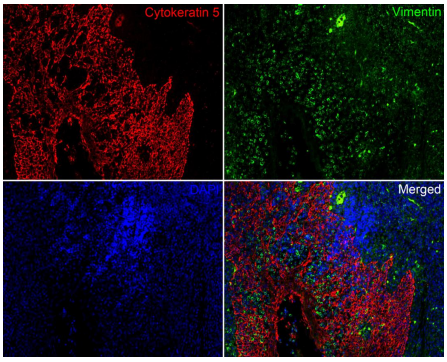

Fig9: Immunofluorescence analysis of paraffin-embedded human tonsil tissue labeling Cytokeratin 5 (ET1610-43) at 1/200 dilution and Vimentin (EM0401) at 1/200 dilution.
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies Cytokeratin 5 (ET1610-43, red) at 1/200 dilution and Vimentin (EM0401, green) at 1/200 dilution at +4℃ overnight, washed with PBS. iFluorTM 594 conjugate-Goat anti-Rabbit IgG (HA1122) and iFluorTM 488 conjugate-Goat anti-Mouse IgG (HA1125)were used as the secondary antibodies at 1/1000 dilution. DAPI was used as nuclear counterstain.

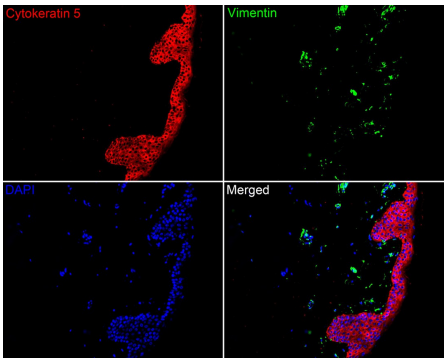

Fig10: Immunofluorescence analysis of paraffin-embedded human skin tissue labeling Cytokeratin 5 (ET1610-43) at 1/200 dilution and Vimentin (EM0401) at 1/200 dilution.
The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS. And then probed with the primary antibodies Cytokeratin 5 (ET1610-43, red) at 1/200 dilution and Vimentin (EM0401, green) at 1/200 dilution at +4℃ overnight, washed with PBS. iFluorTM 594 conjugate-Goat anti-Rabbit IgG (HA1122) and iFluorTM 488 conjugate-Goat anti-Mouse IgG (HA1125)were used as the secondary antibodies at 1/1000 dilution. DAPI was used as nuclear counterstain.

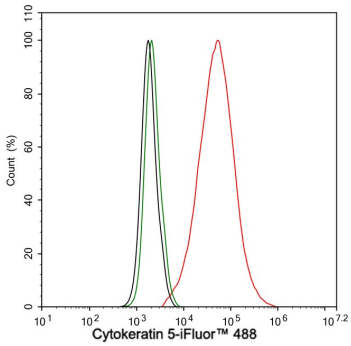

Fig11: Flow cytometric analysis of A431 cells labeling Cytokeratin 5.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1610-43, 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).

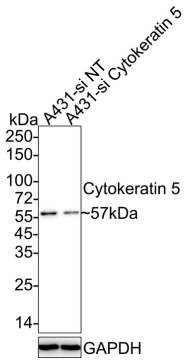

Fig12: Western blot analysis of Cytokeratin 5 on different lysates with Rabbit anti-Cytokeratin 5 antibody (ET1610-43) at 1/5,000 dilution.

Lane 1: A431-si NT cell lysate
Lane 2: A431-si Cytokeratin 5 cell lysate

Lysates/proteins at 10 μg/Lane.

Predicted band size: 62 kDa
Observed band size: 57 kDa

Exposure time: 5 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 (ET1610-43) at 1/5,000 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.

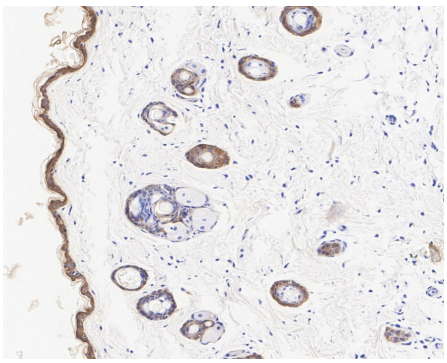

Fig13: Immunohistochemical analysis of paraffin-embedded mouse skin tissue with Rabbit anti-Cytokeratin 5 antibody (ET1610-43) at 1/1,000 dilution.

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 (ET1610-43) 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.

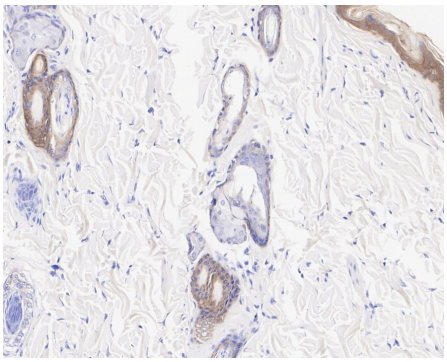

Fig14: Immunohistochemical analysis of paraffin-embedded rat skin tissue with Rabbit anti-Cytokeratin 5 antibody (ET1610-43) at 1/1,000 dilution.

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 (ET1610-43) 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.

Background References

1. Colopy SA et al. A population of progenitor cells in the basal and intermediate layers of the murine bladder urothelium contributes to urothelial development and regeneration. Dev Dyn 243:988-98 (2014).
2. Wang J et al. Symmetrical and asymmetrical division analysis provides evidence for a hierarchy of prostate epithelial cell lineages. Nat Commun 5:4758 (2014).