Anti-Cytokeratin 18 Antibody [SZ80-07]

ET1603-8

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, mIHC
Molecular Wt:	Predicted band size: 48 kDa
Clone number:	SZ80-07

Description: Cytokeratins comprise a diverse group of intermediate filament proteins (IFPs) that are expressed as pairs in both keratinized and non-keratinized epithelial tissue. Cytokeratins play a critical role in differentiation and tissue specialization and function to maintain the overall structural integrity of epithelial cells. Cytokeratins have been found to be useful markers of tissue differentiation which is directly applicable to the characterization of malignant tumors. For example, Cytokeratins 10 and 13 are expressed highly in a subset of squamous cell carcinomas while Cytokeratin 18 is expressed in a majority of adenocarcinomas and basal cell carcinomas. Cytokeratin 18 contains two major phosphorylation sites on Ser 33 and Ser 52. Phosphorylation of Ser 18 is essential for the association of Cytokeratin 18 with 14-3-3 proteins and is involved in keratin organization and distribution.

Immunogen: Recombinant protein within mouse Cytokeratin 18 aa 200-423.

Positive control: A431 cell lysate, HeLa cell lysate, HT-29 cell lysate, mouse kidney, rat skin tissue, Hela, A431, HepG2, human colon carcinoma tissue, human breast carcinoma tissue, human colon tissue,human liver tissue, human stomach tissue, human stomach carcinoma tissue, MCF-7.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P05783 Human | P05784 Mouse | Q5BJY9 Rat

Recommended Dilutions:

WB	1:5,000-1:20,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:50-1:200
FC	1:50-1:100
mIHC	1:3,000-1:10,000

Storage Buffer: 1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

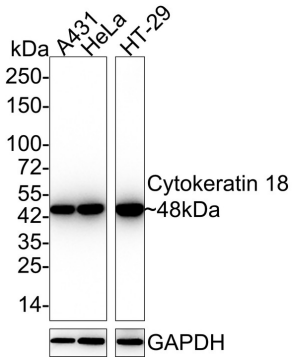

Fig1: Western blot analysis of Cytokeratin 18 on different lysates with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/5,000 dilution.

Lane 1: A431 cell lysate
Lane 2: HeLa cell lysate
Lane 3: HT-29 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 48 kDa
Observed band size: 48 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 (ET1603-8) 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.

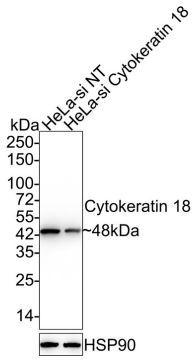

Fig2: Western blot analysis of Cytokeratin 18 on different lysates with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/20,000 dilution.

Lane 1: HeLa-si NT cell lysate
Lane 2: HeLa-si Cytokeratin 18 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 48 kDa
Observed band size: 48 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 (ET1603-8) at 1/20,000 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.

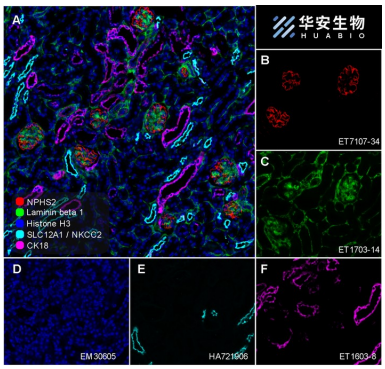

Fig3: Fluorescence multiplex immunohistochemical analysis of mouse kidney (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-NPHS2 (ET7107-34, Red), anti-Laminin beta 1 (ET1703-14, Green), anti-Histone H3 (EM30605, Blue), anti-SLC12A1 / NKCC2 (HA721906, Cyan) and anti-CK18 (ET1603-8, Magenta) on kidney. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in five rounds of staining: in the order of ET7107-34 (1/1,000 dilution), ET1703-14 (1/1,000 dilution), EM30605 (1/500 dilution), HA721906 (1/3,000 dilution) and ET1603-8 (1/3,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. Image acquisition was performed with Olympus VS200 Slide Scanner.

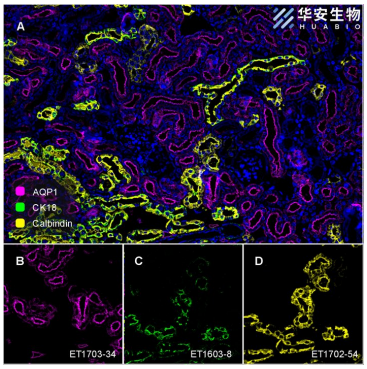

Fig4: Fluorescence multiplex immunohistochemical analysis of mouse kidney (Formalin/PFA-fixed paraffin-embedded sections). Panel A: the merged image of anti-AQP1 (ET1703-34, Violet), anti-CK18 (ET1603-8, Green) and anti-Calbindin (ET1702-54, Yellow) on kidney. HRP Conjugated UltraPolymer Goat Polyclonal Antibody HA1119/HA1120 was used as a secondary antibody. The immunostaining was performed with the Sequential Immuno-staining Kit (IRISKit™MH010101, www.luminiris.cn). The section was incubated in three rounds of staining: in the order of ET1703-34 (1/5,000 dilution), ET1603-8 (1/3,000 dilution) and ET1702-54 (1/4,000 dilution) for 20 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 30 mins at 95℃. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Zeiss Observer 7 Inverted Fluorescence Microscope.

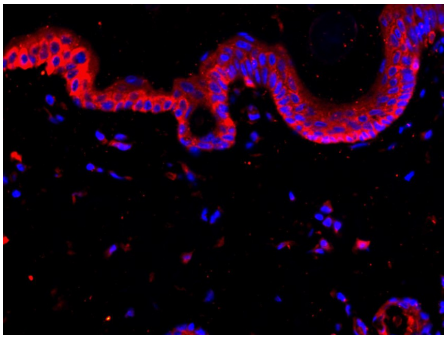

Fig5: Immunofluorescence analysis of paraffin-embedded rat skin tissue labeling Cytokeratin 18 with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/200 dilution.

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 antibody (ET1603-8, red) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 594, HA1122) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

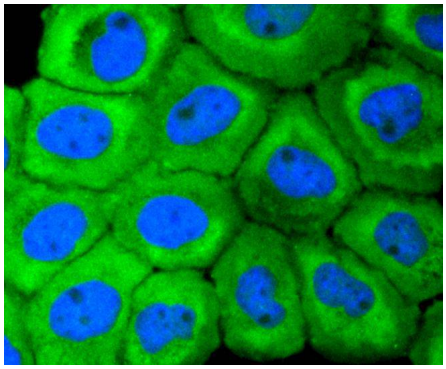

Fig6: Immunocytochemistry analysis of A431 cells labeling Cytokeratin 18 with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/100 dilution.

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 (ET1603-8, 1/100) 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).

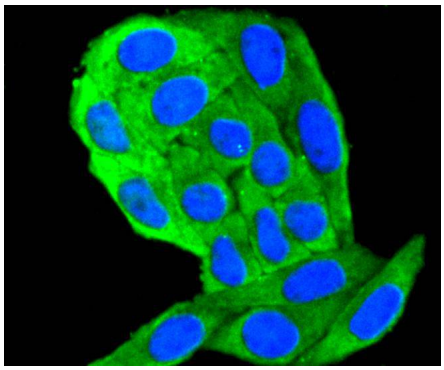

Fig7: Immunocytochemistry analysis of HepG2 cells labeling Cytokeratin 18 with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/100 dilution.

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 (ET1603-8, 1/100) 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).

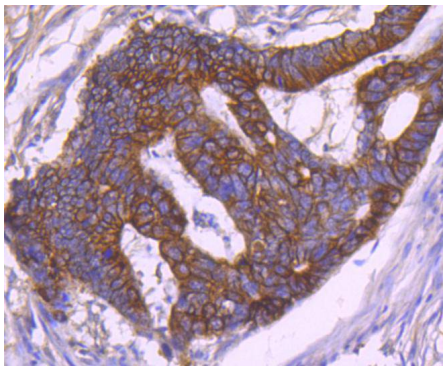

Fig8: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/50 dilution.

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 (ET1603-8, 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.

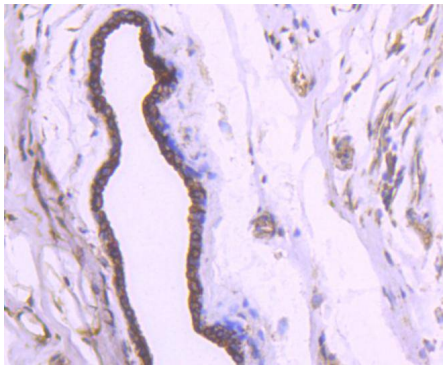

Fig9: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/200 dilution.

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 (ET1603-8, 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.

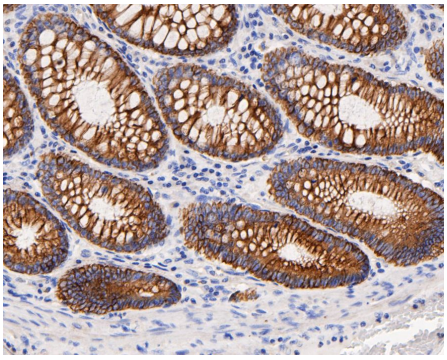

Fig10: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/200 dilution.

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 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-8, 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.

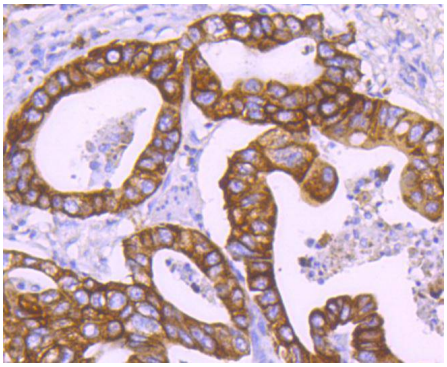

Fig11: Immunohistochemical analysis of paraffin-embedded human stomach tissue with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/200 dilution.

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 (ET1603-8, 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.

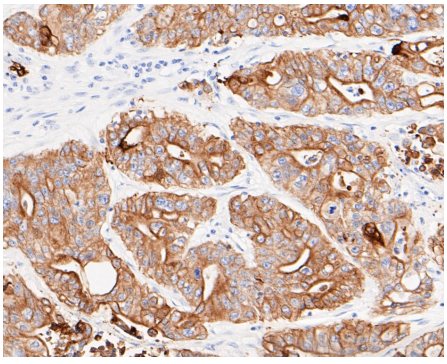

Fig12: Immunohistochemical analysis of paraffin-embedded human stomach carcinoma tissue with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/200 dilution.

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 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-8, 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.

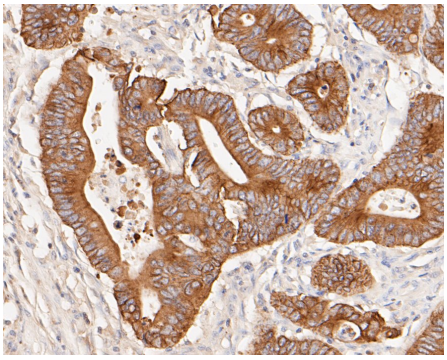

Fig13: Immunohistochemical analysis of paraffin-embedded human colon carcinoma with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/200 dilution.

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 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-8, 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.

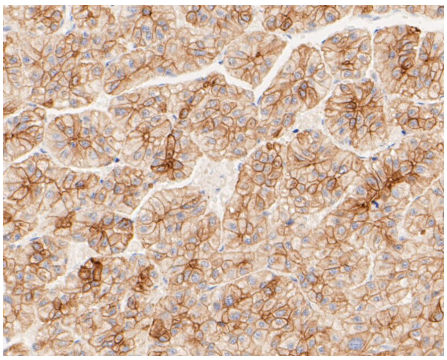

Fig14: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/200 dilution.

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 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1603-8, 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.

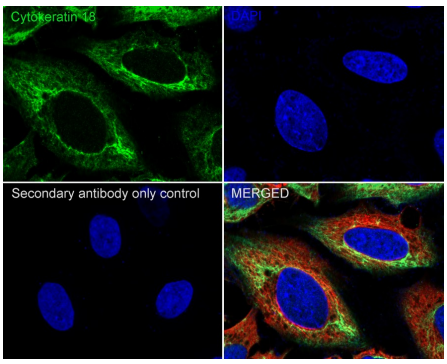

Fig15: Immunocytochemistry analysis of HeLa cells labeling Cytokeratin 18 with Rabbit anti-Cytokeratin 18 antibody (ET1603-8) at 1/100 dilution.

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 18 antibody (ET1603-8) 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.

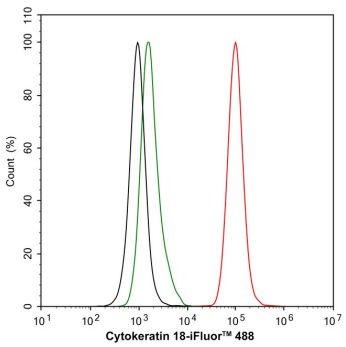

Fig16: Flow cytometric analysis of HeLa cells labeling Cytokeratin 18.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1603-8, 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).

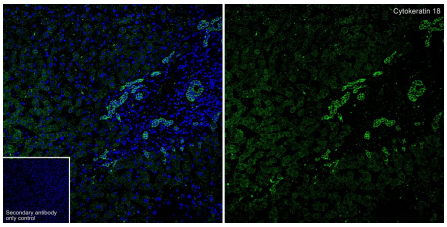

Fig17: Application: IF-Tissue

Species: Human

Site: liver

Sample: Paraffin-embedded section

Antibody concentration: 1/200

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

Background References

1. Kuramoto G et al. Preventive effect of oral mucosal epithelial cell sheets on intrauterine adhesions. Hum Reprod 30:406-16 (2015).
2. Huang YS et al. RGD surface functionalization of the hydrophilic acrylic intraocular lens material to control posterior capsular opacification. PLoS One 9:e114973 (2014).