Anti-Cytokeratin 5+6 Antibody [PSH0-68] - BSA and Azide free

HA750645

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 60 kDa
Clone number:	PSH0-68

Description: Cytokeratins comprise a diverse group of intermediate filament proteins (IFPs) that are expressed as 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 alpha-helical coiled-coil dimers associate laterally end-to-end to form 10 nm diameter filaments. Cytokeratins, which are useful markers of tissue differentiation, also aid in the characterization of malignant tumors. IL-1 and TNFα induce transcription of cytokeratin 6 in epidermal keratinocytes via the C/EBP β transcription factor. In humans, multiple isoforms of cytokeratin 6 (6A-6F), encoded by several highly homologous genes, have distinct tissue expression patterns, and cytokeratin 6A is the dominant form in epithelial tissue. The gene encoding human cytokeratin 6A maps to chromosome 12q13, and mutations in this gene are linked to several inheritable hair and skin pathologies.

Immunogen: Synthetic peptide within human Cytokeratin 6 186-235.

Positive control: A431 cell lysate, NCI-H226 cell lysate, mouse brain tissue lysate, rat brain tissue lysate, HeLa, A431.

Subcellular location: Cytoskeleton.

Database links: SwissProt: P02538 Human | P04259 Human | P13647 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
FC	1:500-1:1,000

Storage Buffer: 1*PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

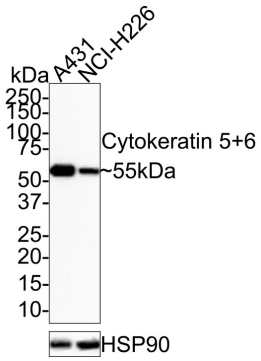

Fig1: Western blot analysis of Cytokeratin 5+6 on different lysates with Rabbit anti-Cytokeratin 5+6 antibody (HA750645) at 1/1,000 dilution.

Lane 1: A431 cell lysate
Lane 2: NCI-H226 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 60 kDa
Observed band size: 55 kDa

Exposure time: 1 minute;

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 (HA750645) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

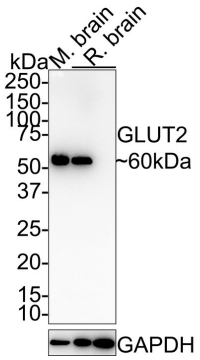

Fig2: Western blot analysis of Cytokeratin 5+6 on different lysates with Rabbit anti-Cytokeratin 5+6 antibody (HA750645) at 1/1,000 dilution.

Lane 1: Mouse brain tissue lysate (hot lysis)
Lane 2: Rat brain tissue lysate (hot lysis)
Lane 3: Rat brain tissue lysate (RIPA lysis)

Lysates/proteins at 40 µg/Lane.

Predicted band size: 60 kDa
Observed band size: 60 kDa

Exposure time: 1 minute 40 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 (HA750645) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

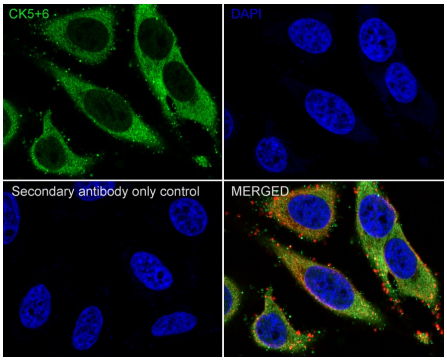

Fig3: Immunocytochemistry analysis of HeLa cells labeling Cytokeratin 5+6 with Rabbit anti-Cytokeratin 5+6 antibody (HA750645) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Cytokeratin 5+6 antibody (HA750645) at 1/100 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/200 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

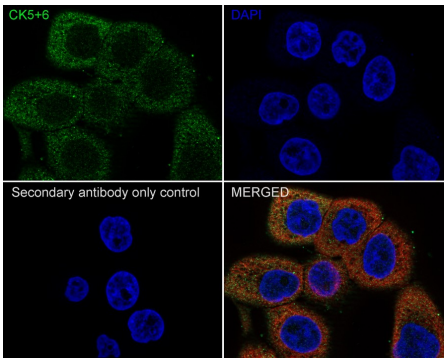

Fig4: Immunocytochemistry analysis of A431 cells labeling Cytokeratin 5+6 with Rabbit anti-Cytokeratin 5+6 antibody (HA750645) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Cytokeratin 5+6 antibody (HA750645) at 1/100 dilution in 2% negative goat serum overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/200 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

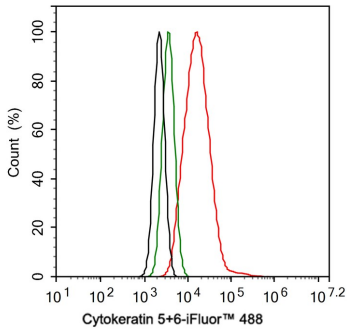

Fig5: Flow cytometric analysis of A431 cells labeling Cytokeratin 5+6.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA750645, 1ug/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).

Background References

1. Yamamoto Y et al. Cytokeratin 5/6 expression in pT1 bladder cancer predicts intravesical recurrence in patients treated with bacillus Calmette-Guérin instillation. Pathology. 2022 Oct
2. Iakymenko OA et al. Utility of D2-40, Cytokeratin 5/6, and High-Molecular-weight Cytokeratin (Clone 34βE12) in Distinguishing Intraductal Spread of Urothelial Carcinoma From Prostatic Stromal Invasion. Am J Surg Pathol. 2022 Apr