Anti-S100A8 Antibody [PSH09-70]

HA723135

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, IF-Cell, FC, IF-Tissue, IP
Molecular Wt:	Predicted band size: 11 kDa
Clone number:	PSH09-70

Description: S100 calcium-binding protein A8 (S100A8) is a protein that in humans is encoded by the S100A8 gene.It is also known as calgranulin A.The proteins S100A8 and S100A9 form a heterodimer called calprotectin.The protein encoded by this gene is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. S100 proteins are localized in the cytoplasm and/or nucleus of a wide range of cells, and involved in the regulation of a number of cellular processes such as cell cycle progression and differentiation. S100 genes include at least 13 members which are located as a cluster on chromosome 1q21. This protein may function in the inhibition of casein kinase and as a cytokine. Altered expression of this protein is associated with the disease cystic fibrosis and post COVID-19 condition.

Immunogen: Recombinant protein within human S100A8 aa 1-93/93

Positive control: SK-Br-3 cell lysate, human breast carcinoma tissue, human liver tissue, human spleen tissue, human tonsil tissue, SK-Br-3.

Subcellular location: Secreted; Cytoplasm; Cytoplasm, cytoskeleton; Cell membrane.

Database links: SwissProt: P05109 Human

Recommended Dilutions:

WB	1:2,000-1:5,000
IHC-P	1:10,000
IF-Cell	1:50,000
FC	1:1,000
IF-Tissue	1:1,000-1:3,000
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

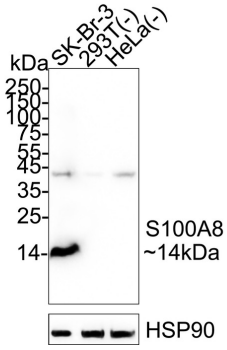

Fig1: Western blot analysis of S100A8 on different lysates with Rabbit anti-S100A8 antibody (HA723135) at 1/5,000 dilution.

Lane 1: SK-Br-3 cell lysate (20 µg/Lane)
Lane 2: 293T cell lysate (negative) (20 µg/Lane)
Lane 3: HeLa cell lysate (negative) (20 µg/Lane)

Predicted band size: 11 kDa
Observed band size: 14 kDa

Exposure time: 20 seconds; ECL: K1801;

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 (HA723135) 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.

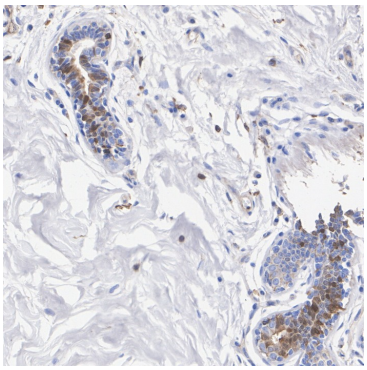

Fig2: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-S100A8 antibody (HA723135) at 1/10,000 dilution.

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 (HA723135) at 1/10,000 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.

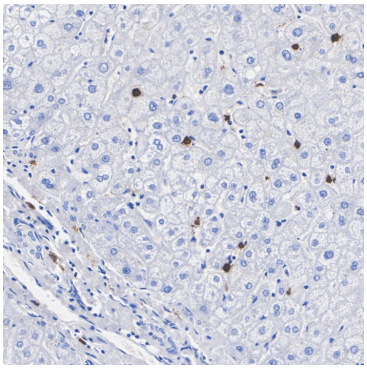

Fig3: Immunohistochemical analysis of paraffin-embedded human liver tissue with Rabbit anti-S100A8 antibody (HA723135) at 1/10,000 dilution.

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 (HA723135) at 1/10,000 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.

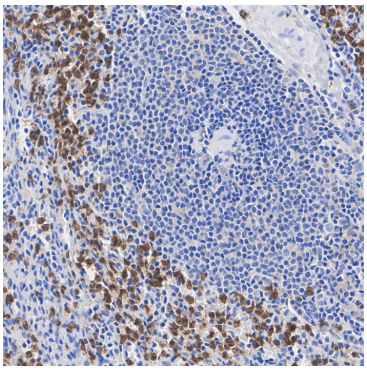

Fig4: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-S100A8 antibody (HA723135) at 1/10,000 dilution.

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 (HA723135) at 1/10,000 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.

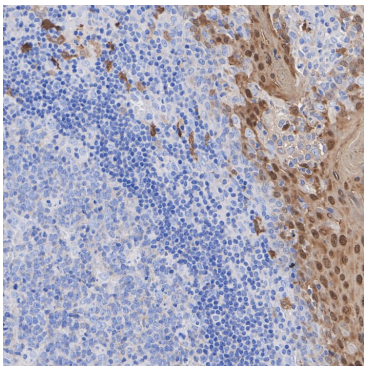

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-S100A8 antibody (HA723135) at 1/10,000 dilution.

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 (HA723135) at 1/10,000 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.

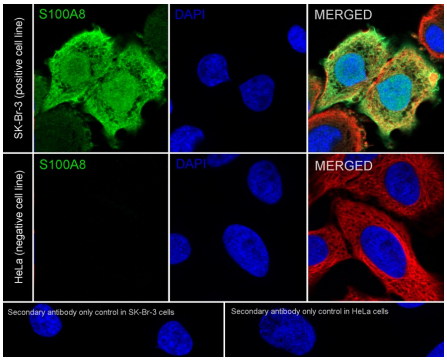

Fig6: Immunocytochemistry analysis of SK-Br-3 (positive) and HeLa (negative) labeling S100A8 with Rabbit anti-S100A8 antibody (HA723135) at 1/50,000 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-S100A8 antibody (HA723135) at 1/50,000 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 (HA601187, 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.

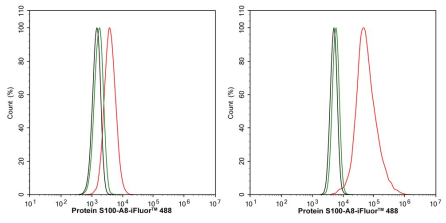

Fig7: Flow cytometric analysis of 293T (left, negative) and SK-Br-3 (right, positive) cells labeling S100A8.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723135, 1/1,000) (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).

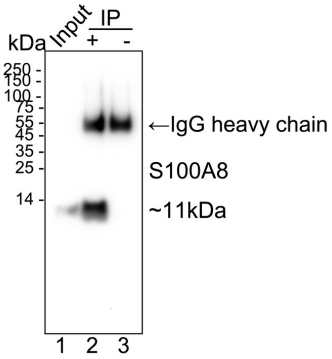

Fig8: S100A8 was immunoprecipitated from 0.2 mg SK-Br-3 cell lysate with HA723135 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA723135 at 1/1,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: SK-Br-3 cell lysate (input)
Lane 2: HA723135 IP in SK-Br-3 cell lysate
Lane 3: Rabbit IgG instead of HA723135 in SK-Br-3 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 minutes; ECL: K1801

Background References

1. Chen Y, Ouyang Y, Li Z, Wang X, Ma J. S100A8 and S100A9 in Cancer. Biochim Biophys Acta Rev Cancer. 2023 May;1878(3):188891. doi: 10.1016/j.bbcan.2023.188891. Epub 2023 Mar 29. PMID: 37001615.
2. Wang S, Song R, Wang Z, Jing Z, Wang S, Ma J. S100A8/A9 in Inflammation. Front Immunol. 2018 Jun 11;9:1298. doi: 10.3389/fimmu.2018.01298. PMID: 29942307; PMCID: PMC6004386.