Anti-Mesothelin Antibody [PSH02-30]

HA721810

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P
Molecular Wt:	Predicted band size: 68 kDa
Clone number:	PSH02-30

Description: This gene encodes a preproprotein that is proteolytically processed to generate two protein products, megakaryocyte potentiating factor and mesothelin. Megakaryocyte potentiating factor functions as a cytokine that can stimulate colony formation of bone marrow megakaryocytes. Mesothelin is a glycosylphosphatidylinositol-anchored cell-surface protein that may function as a cell adhesion protein. This protein is overexpressed in epithelial mesotheliomas, ovarian cancers and in specific squamous cell carcinomas. Alternative splicing results in multiple transcript variants, at least one of which encodes an isoform that is proteolytically processed.

Immunogen: Recombinant protein within human Mesothelin aa 273-622 / 622 (Q13421-3).

Positive control: SiHa cell lysate, NCI-H226 cell lysate, HeLa cell lysate, NIH:OVCAR-3 cell lysate, NCI-H226, human ovarian carcinoma tissue, human tonsil tissue.

Subcellular location: Cell membrane, Golgi apparatus; Secreted.

Database links: SwissProt: Q13421 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
IF-Tissue	1:100
IHC-P	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% 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

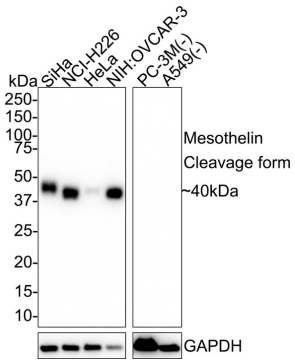

Fig1: Western blot analysis of Mesothelin on different lysates with Rabbit anti-Mesothelin antibody (HA721810) at 1/1,000 dilution.

Lane 1: SiHa cell lysate
Lane 2: NCI-H226 cell lysate
Lane 3: HeLa cell lysate
Lane 4: NIH:OVCAR-3 cell lysate
Lane 5: PC-3M cell lysate (negative)
Lane 6: A549 cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 68 kDa
Observed band size: 40 kDa

Exposure time: 1 minute 2 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 (HA721810) 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 1/50,000 dilution was used for 1 hour at room temperature.

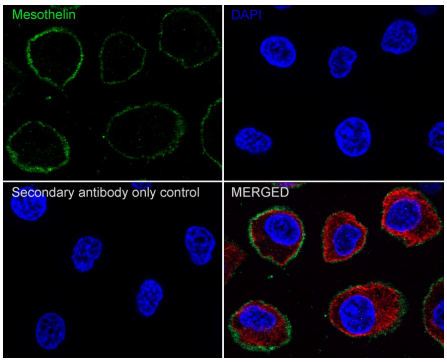

Fig2: Immunocytochemistry analysis of NCI-H226 cells labeling Mesothelin with Rabbit anti-Mesothelin antibody (HA721810) at 1/100 dilution.

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-Mesothelin antibody (HA721810) 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.

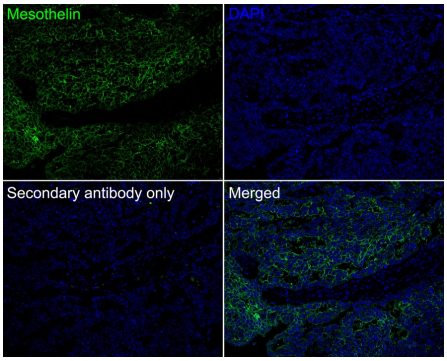

Fig3: Immunofluorescence analysis of paraffin-embedded human ovarian carcinoma tissue labeling Mesothelin with Rabbit anti-Mesothelin antibody (HA721810) at 1/100 dilution.

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 (HA721810, green) at 1/100 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

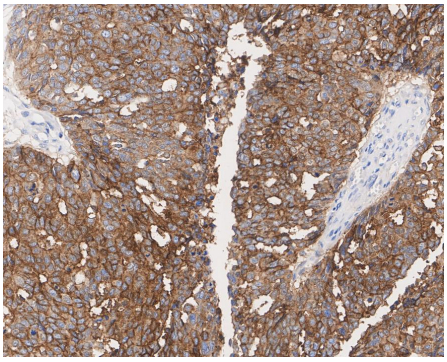

Fig4: Immunohistochemical analysis of paraffin-embedded human ovarian carcinoma tissue with Rabbit anti-Mesothelin antibody (HA721810) at 1/1,000 dilution.

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 (HA721810) 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.

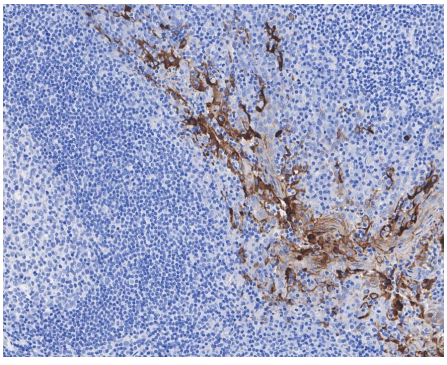

Fig5: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-Mesothelin antibody (HA721810) at 1/1,000 dilution.

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 (HA721810) 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.

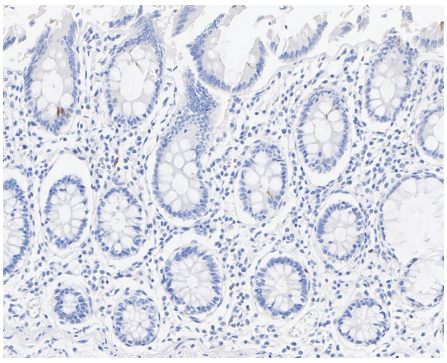

Fig6: Immunohistochemical analysis of paraffin-embedded human colon tissue (negative control) with Rabbit anti-Mesothelin antibody (HA721810) at 1/1,000 dilution.

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 (HA721810) 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.

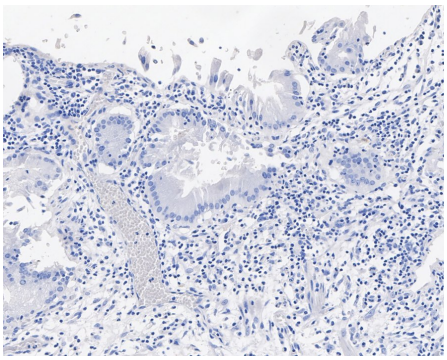

Fig7: Immunohistochemical analysis of paraffin-embedded human gallbladder tissue (negative control) with Rabbit anti-Mesothelin antibody (HA721810) at 1/1,000 dilution.

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 (HA721810) 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. Klampatsa A et al. Mesothelin-targeted CAR-T cell therapy for solid tumors. Expert Opin Biol Ther. 2021 Apr
2. Schoutrop E et al. Mesothelin-Specific CAR T Cells Target Ovarian Cancer. Cancer Res. 2021 Jun