Anti-MUC16 Antibody [PSH21-77]

HA724248

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 1519 kDa
Clone number:	PSH21-77

Description: Mucin-16 (MUC-16) also known as Ovarian cancer-related tumor marker CA125 is a protein that in humans is encoded by the MUC16 gene. MUC-16 is a member of the mucin family glycoproteins. MUC-16 has found application as a tumor marker or biomarker that may be elevated in the blood of some patients with specific types of cancers, most notably ovarian cancer, or other conditions that are benign. MUC16 is a component of the ocular surface (including the cornea and conjunctiva), the respiratory tract and the female reproductive tract epithelia. Since MUC16 is highly glycosylated it creates a hydrophilic environment that acts as a lubricating barrier against foreign particles and infectious agents on the apical membrane of epithelial cells. Also, the cytoplasmic tail of MUC16 has been shown to interact with cytoskeleton by binding members of the ERM protein family. The expression of mucin 16 has been shown to be altered in dry eye, cystic fibrosis, and several types of cancers.

Immunogen: Recombinant protein within human MUC16 aa 13,302-14,451.

Positive control: NIH:OVCAR-3 cell lysate, HeLa cell lysate, human ovarian carcinoma tissue, human salivary glands tissue, NIH:OVCAR-3.

Subcellular location: Cell membrane, secreted, extracellular space.

Database links: SwissProt: Q8WXI7 Human

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:500
IHC-P	1:200-1:1,000
FC	1:1,000
IP	1-2μg/sample

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

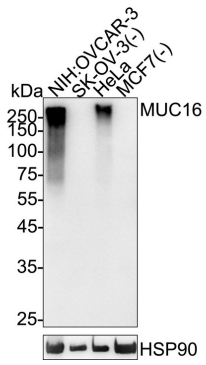

Fig1: Western blot analysis of MUC16 on different lysates with Rabbit anti-MUC16 antibody (HA724248) at 1/5,000 dilution.

Lane 1: NIH:OVCAR-3 cell lysate
Lane 2: SK-OV-3 cell lysate (negative)
Lane 3: HeLa cell lysate
Lane 4: MCF7 cell lysate (negative)

Lysates/proteins at 15 µg/Lane.

Predicted band size: 1519 kDa

Exposure time: 59 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 (HA724248) at 1/5,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

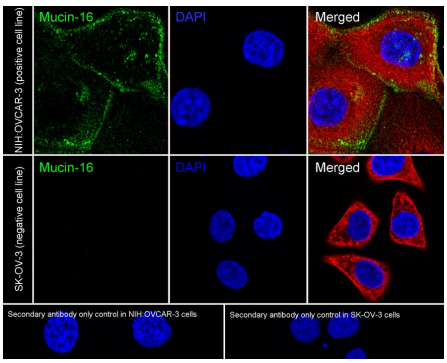

Fig2: Immunocytochemistry analysis of NIH:OVCAR-3 (positive) and SK-OV-3 (negative) labeling MUC16 with Rabbit anti-MUC16 antibody (HA724248) at 1/500 dilution.

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-MUC16 antibody (HA724248) at 1/500 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.

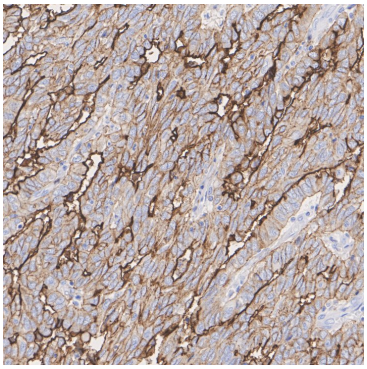

Fig3: Immunohistochemical analysis of paraffin-embedded human ovarian carcinoma tissue with Rabbit anti-MUC16 antibody (HA724248) at 1/1,000 dilution.

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 (HA724248) 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.

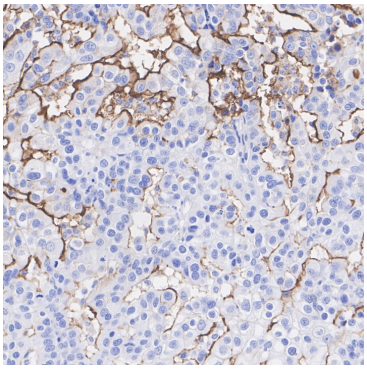

Fig4: Immunohistochemical analysis of paraffin-embedded human ovarian carcinoma tissue with Rabbit anti-MUC16 antibody (HA724248) at 1/1,000 dilution.

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 (HA724248) 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.

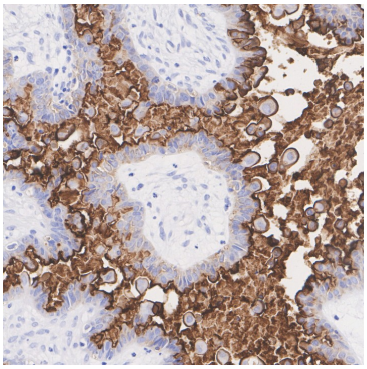

Fig5: Immunohistochemical analysis of paraffin-embedded human ovarian carcinoma tissue with Rabbit anti-MUC16 antibody (HA724248) at 1/1,000 dilution.

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 (HA724248) 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.

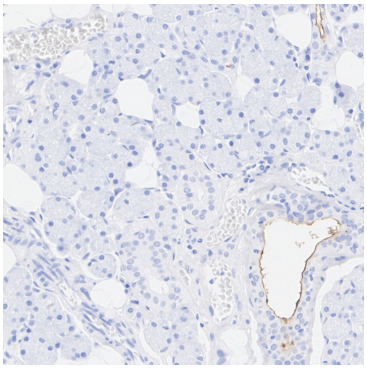

Fig6: Immunohistochemical analysis of paraffin-embedded human salivary glands tissue with Rabbit anti-MUC16 antibody (HA724248) at 1/200 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA724248) at 1/200 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.

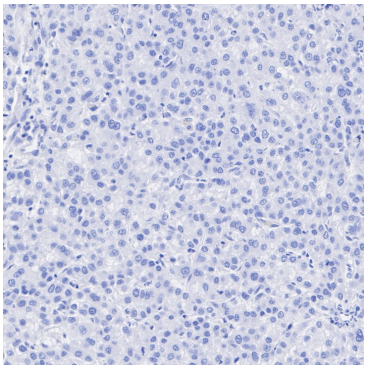

Fig7: Immunohistochemical analysis of paraffin-embedded human hepatocellular carcinoma tissue with Rabbit anti-MUC16 antibody (HA724248) at 1/200 dilution.

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 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA724248) at 1/200 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.

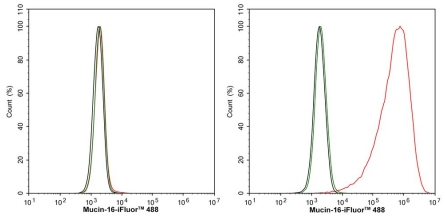

Fig8: Flow cytometric analysis of SK-OV-3 (left, negative) and NIH:OVCAR-3 (right, positive) cells labeling MUC16.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (HA724248, 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).

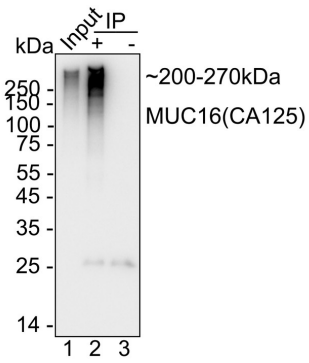

Fig9: MUC16 was immunoprecipitated from 0.2 mg NIH:OVCAR-3 cell lysate with HA724248 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA724248 at 1/5,000 dilution. HRP Conjugated Anti-Rabbit IgG for IP Nano-secondary antibody at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: NIH:OVCAR-3 cell lysate (input)
Lane 2: HA724248 IP in NIH:OVCAR-3 cell lysate
Lane 3: Rabbit IgG instead of HA724248 in NIH:OVCAR-3 cell lysate

Blocking/Dilution buffer: primary antibody dilution (K1803)
Exposure time: 3 seconds; ECL: K1801

Background References

1. Giamougiannis P et al. The evolving role of MUC16 (CA125) in the transformation of ovarian cells and the progression of neoplasia. Carcinogenesis. 2021 Apr
2. Wang F et al. MUC16 promotes EOC proliferation by regulating GLUT1 expression. J Cell Mol Med. 2021 Mar