Anti-TROP2 Antibody [A5E2]

HA600068



Product Type: Mouse monoclonal IgG1, primary antibodies

Species reactivity: Human

Applications: WB, IF-Cell, IHC-P, FC

Molecular Wt: Predicted band size: 36 kDa

Clone number: A5E2

Description: TROP-2, also known as tumor-associated calcium signal transducer 2 (TACSTD2),

pancreatic carcinoma marker protein GA733-1, membrane component chromosome 1, surface marker 1 (M1S1) or epithelial glycoprotein-1 (EGP-1), is a cell surface glycoprotein receptor. It is a single pass type I membrane protein containing one thryoglobulin type-1 domain, an epidermal growth factor-like repeat, a phosphatidylinositol binding site and tyrosine phosphorylation sites near the C-terminus. TROP-2 plays a role in tranducing intracellular calcium signals. It is expressed in trophoblast cells, cornea and multistratified epithelia. It is also highly expressed in several types of tumors and is involved in regulating the growth of carcinoma cells. Mutations in the gene encoding TROP-2 can result in gelatinous drop-like corneal dystrophy (GDLD) also referred to as lattice corneal dystrophy

type III, an autosomal recessive disorder that causes severe visual impairment.

Immunogen: Recombinant protein within human TROP2 aa 1-274 / 323 (Extracellular).

Positive control: MCF7 cell lysate, A431 cell lysate, A431, human skin tissue, human breast cancer tissue,

human esophagus tissue, MDA-MB-468.

Subcellular location: Membrane.

Database links: SwissProt: P09758 Human

Recommended Dilutions:

WB 1:2,000 IF-Cell 1:100

IHC-P 1:400-1:1,000 **FC** 1:1,000

Storage Buffer: 1*TBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4° C. Store at $+4^{\circ}$ C short term (1-2 weeks). It is recommended to aliquot into

single-use upon delivery. Store at -20 °C long term.

Purity: Protein G affinity purified.

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Images

kDa 1675 Herc Loss (rendering)

250 - 180 - 75 - 55 - 45 - 35 - 25 - 14 - 36 - GAPDH

Fig1: Western blot analysis of TROP2 on different lysates with Mouse anti-TROP2 antibody (HA600068) at 1/2,000 dilution.

Lane 1: MDA-MB-468 cell lysate

Lane 2: MCF7 cell lysate

Lane 3: A549 cell lysate (negative control)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 36 kDa Observed band size: 40-60 kDa

Exposure time: 16 seconds;

4-20% SDS-PAGE gel.

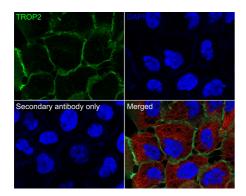


Fig2: Immunocytochemistry analysis of A431 cells labeling TROP2 with Mouse anti-TROP2 antibody (HA600068) at 1/100 dilution.

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 Mouse anti-TROP2 antibody (HA600068) at 1/100 dilution in 1% BSA in PBST overnight at 4 $^{\circ}$ C. Goat Anti-Mouse IgG H&L (iFluor 488, HA1125) 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 (ET1602-4, red) was stained at 1/100 dilution overnight at $+4^{\circ}$ C. Goat Anti-Rabbit IgG H&L (iFluor 594, HA1122) were used as the secondary antibody at 1/1,000 dilution.

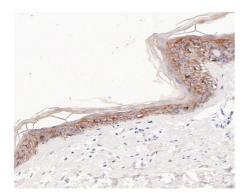


Fig3: Immunohistochemical analysis of paraffin-embedded human skin tissue using anti-TROP2 antibody. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA600068, 1/400) 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.

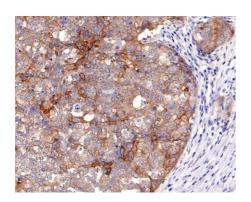


Fig4: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue using anti-TROP2 antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA600068, 1/800) 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.

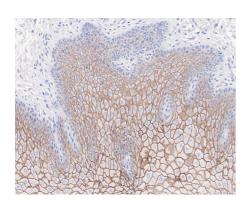


Fig5: Immunohistochemical analysis of paraffin-embedded human esophagus tissue with Mouse anti-TROP2 antibody (HA600068) at 1/1,000 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (HA600068) 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.

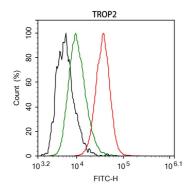


Fig6: Flow cytometric analysis of TROP2 was done on MDA-MB-468 cells. The cells were fixed, permeabilized and stained with the primary antibody (HA600068, 1ug/ml) (red) compared with Mouse IgG, monoclonal - Isotype Control (green). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Mouse IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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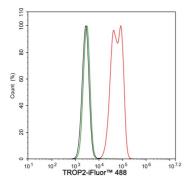


Fig7: Flow cytometric analysis of A431 cells labeling TROP2.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (HA600068, $1\mu g/mL$) (red) compared with Mouse IgG1 Isotype Control (green). After incubation of the primary antibody at +4°C for 30 minutes, the cells were stained with a iFluor 488 conjugate-Goat anti-Mouse IgG Secondary antibody (HA1125) at 1/1,000 dilution for 30 minutes at +4°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

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

Background References

- 1. Lenárt S. et. al. Trop2: Jack of All Trades, Master of None. Cancers (Basel). 2020 Nov
- 2. Hsu EC. et. al. Trop2 is a driver of metastatic prostate cancer with neuroendocrine phenotype via PARP1. Proc Natl Acad Sci U S A. 2020 Jan