Anti-Scramblase 1 Antibody [JE35-30]

HA722327

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 35 kDa
Clone number:	JE35-30

Description: Scramblase is a protein responsible for the translocation of phospholipids between the two monolayers of a lipid bilayer of a cell membrane. In humans, phospholipid scramblases (PLSCRs) constitute a family of five homologous proteins that are named as hPLSCR1–hPLSCR5. Phospholipid scramblase 1 (PLSCR1), a lipid-binding protein that enters the nucleus via the nonclassical NLS (257)GKISKHWTGI(266). The structure of the nuclear localisation sequence of scramblase PLSCR1 complexed to importin was determined using X-ray diffraction with a resolution of 2.20 Ångströms. It is found in most mammals including humans. The import sequence lacks a continuous stretch of positively charged residues, and it is enriched in hydrophobic residues. Thus, Scramblase can transport negatively charged phospholipids from the inside of the cell to the outside of the cell. The importin structure is composed of many alpha helices that integrate the protein into membranes. The role of importin is to move proteins such as scramblase into the nucleus.

Immunogen: Synthetic peptide within Human Scramblase 1 aa 11-60 / 318.

Positive control: SK-MEL-28 cell lysate, HeLa cell lysate, THP-1 cell lysate, MDA-MB-231 cell lysate, SK-MEL-28, human breast cancer tissue, human spleen tissue.

Subcellular location: Cell membrane, Nucleus, Membrane, Cytoplasm, perinuclear region.

Database links: SwissProt: O15162 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:100
IHC-P	1:200
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

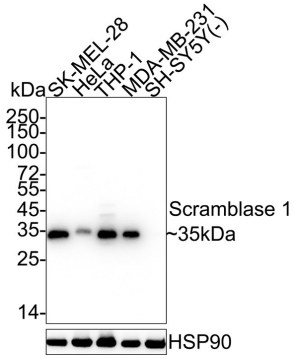

Fig1: Western blot analysis of Scramblase 1 on different lysates with Rabbit anti-Scramblase 1 antibody (HA722327) at 1/1,000 dilution.

Lane 1: SK-MEL-28 cell lysate
Lane 2: HeLa cell lysate
Lane 3: THP-1 cell lysate
Lane 4: MDA-MB-231 cell lysate
Lane 5: SH-SY5Y cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 35 kDa
Observed band size: 35 kDa

Exposure time: 9 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 (HA722327) at 1/1,000 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.

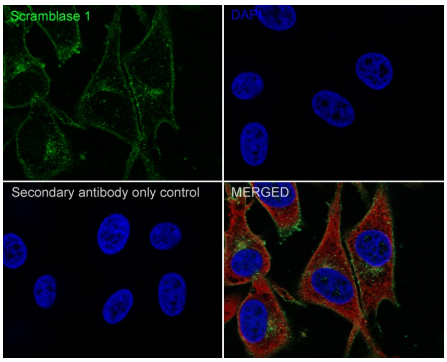

Fig2: Immunocytochemistry analysis of SK-MEL-28 cells labeling Scramblase 1 with Rabbit anti-Scramblase 1 antibody (HA722327) at 1/100 dilution.

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-Scramblase 1 antibody (HA722327) 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.

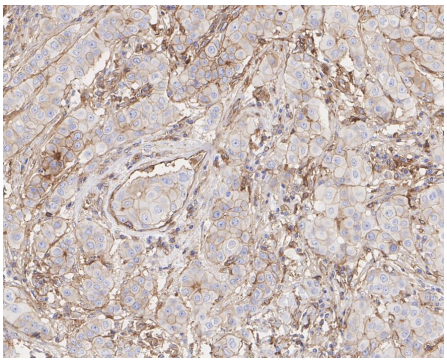

Fig3: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Scramblase 1 antibody (HA722327) at 1/200 dilution.

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 (HA722327) 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.

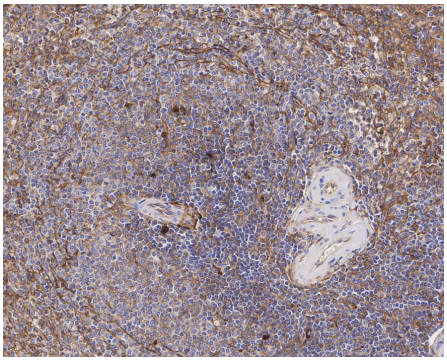

Fig4: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-Scramblase 1 antibody (HA722327) at 1/200 dilution.

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 (HA722327) 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.

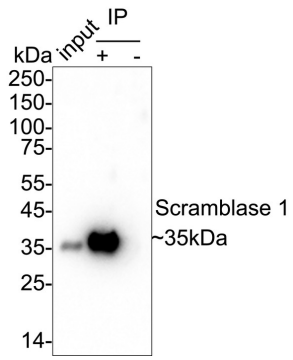

Fig5: Scramblase 1 was immunoprecipitated from 0.2 mg SK-MEL-28 cell lysate with HA722327 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA722327 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: SK-MEL-28 cell lysate (input)
Lane 2: HA722327 IP in SK-MEL-28 cell lysate
Lane 3: Rabbit IgG instead of HA722327 in SK-MEL-28 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 14 seconds; ECL: K1801

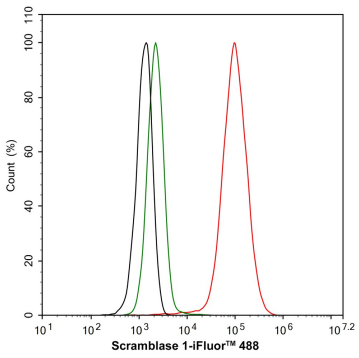

Fig6: Flow cytometric analysis of SK-MEL-28 cells labeling Scramblase 1.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722327, 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).

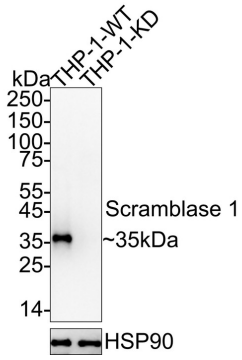

Fig7: Western blot analysis of Scramblase 1 on different lysates with Rabbit anti-Scramblase 1 antibody (HA722327) at 1/5,000 dilution.

Lane 1: THP-1-si NT cell lysate
Lane 2: THP-1-si Scramblase 1 cell lysate

Lysates/proteins at 5 µg/Lane.

Predicted band size: 35 kDa
Observed band size: 35 kDa

Exposure time: 20 seconds; ECL: K1802;

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 (HA722327) 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.

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

Background References

1. Li H. et. al. PLSCR1/IP3R1/Ca(2+) axis contributes to differentiation of primary AML cells induced by wogonoside. Cell Death Dis. 2017 May
2. Li H. et. al. Wogonoside induces depalmitoylation and translocation of PLSCR1 and N-RAS in primary acute myeloid leukaemia cells. J Cell Mol Med. 2018 Apr