Anti-CD63 Antibody [PSH06-64]

HA722731

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC, IP, IHC-P
Molecular Wt:	Predicted band size: 26 kDa
Clone number:	PSH06-64

Description: CD63 antigen is a protein that, in humans, is encoded by the CD63 gene. CD63 is mainly associated with membranes of intracellular vesicles, although cell surface expression may be induced. The protein encoded by this gene is a member of the transmembrane 4 superfamily, also known as the tetraspanin family. Most of these members are cell-surface proteins that are characterized by the presence of four hydrophobic domains. The proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth, and motility. This encoded protein is a cell surface glycoprotein that is known to complex with integrins. It may function as a blood platelet activation marker. Deficiency of this protein is associated with Hermansky-Pudlak Syndrome . Also this gene has been associated with tumor progression. The use of alternate polyadenylation sites has been found for this gene. Alternative splicing results in multiple transcript variants encoding different proteins.

Immunogen: Recombinant protein within

Positive control: SK-MEL-28 cell lysate, U-87 MG cell lysate, HUVEC cell lysate, K-562 cell lysate, HEK-293 cell lysate, HL-60 cell lysate, K-562, human melanoma tissue.

Subcellular location: Cell membrane, Lysosome membrane, Late endosome membrane, Endosome, multivesicular body, Melanosome, Secreted, extracellular exosome, Cell surface.

Database links: SwissProt: P08962 Human

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:250
FC	1:1,000
IP	1-2μg/sample
IHC-P	1:200

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

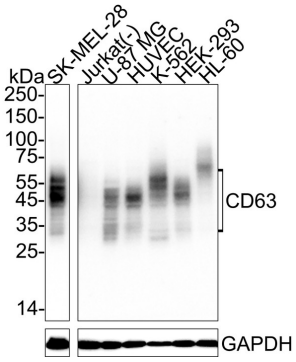

Fig1: Western blot analysis of CD63 on different lysates with Rabbit anti-CD63 antibody (HA722731) at 1/1,000 dilution.

Lane 1: SK-MEL-28 cell lysate
Lane 2: Jurkat cell lysate (negative)
Lane 3: U-87 MG cell lysate
Lane 4: HUVEC cell lysate
Lane 5: K-562 cell lysate
Lane 6: HEK-293 cell lysate
Lane 7: HL-60 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 26 kDa
Observed band size: 30-65 kDa

Exposure time: Lane 1: 4 seconds; Lane 2-7: 18 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 (HA722731) 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.

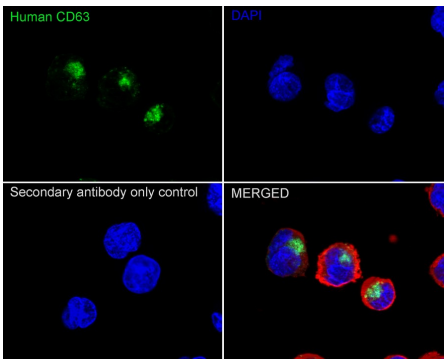

Fig2: Immunocytochemistry analysis of K-562 cells labeling CD63 with Rabbit anti-CD63 antibody (HA722731) at 1/250 dilution.

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-CD63 antibody (HA722731) at 1/250 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.

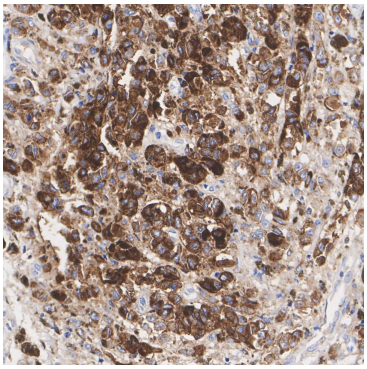

Fig3: Immunohistochemical analysis of paraffin-embedded human melanoma tissue with Rabbit anti-CD63 antibody (HA722731) at 1/200 dilution.

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 (HA722731) 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.

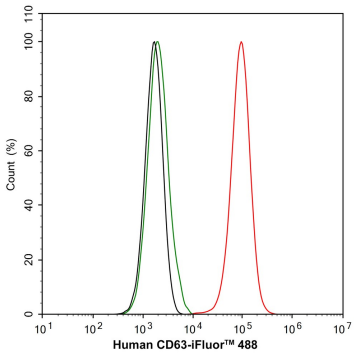

Fig4: Flow cytometric analysis of K-562 cells labeling CD63.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (HA722731, 1µg/mL) (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).

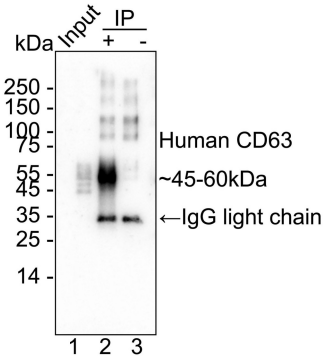

Fig5: CD63 was immunoprecipitated from 0.2 mg SK-MEL-28 cell lysate with HA722731 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA722731 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: SK-MEL-28 cell lysate (input)
Lane 2: HA722731 IP in SK-MEL-28 cell lysate
Lane 3: Rabbit IgG instead of HA722731 in SK-MEL-28 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 minute 31 seconds; ECL: K1801

Background References

1. Mathieu M et al. Specificities of exosome versus small ectosome secretion revealed by live intracellular tracking of CD63 and CD9. Nat Commun. 2021 Jul
2. Yanatori I et al. CD63 is regulated by iron via the IRE-IRP system and is important for ferritin secretion by extracellular vesicles. Blood. 2021 Oct