Anti-CD33 Antibody [PSH02-51]

HA721827

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 40 kDa
Clone number:	PSH02-51

Description: CD33 or Siglec-3 (sialic acid binding Ig-like lectin 3, SIGLEC3, SIGLEC-3, gp67, p67) is a transmembrane receptor expressed on cells of myeloid lineage. It is usually considered myeloid-specific, but it can also be found on some lymphoid cells. It binds sialic acids, therefore is a member of the SIGLEC family of lectins. CD33 can be stimulated by any molecule with sialic acid residues such as glycoproteins or glycolipids. Upon binding, the immunoreceptor tyrosine-based inhibition motif (ITIM) of CD33, present on the cytosolic portion of the protein, is phosphorylated and acts as a docking site for Src homology 2 (SH2) domain-containing proteins like SHP phosphatases. This results in a cascade that inhibits phagocytosis in the cell.

Immunogen: Recombinant protein within human CD33 aa 1-282 / 364 (P20138).

Positive control: TF-1 cell lysate, TF-1, THP-1.

Subcellular location: Cell membrane; Peroxisome.

Database links: SwissProt: P20138 Human

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:500
FC	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

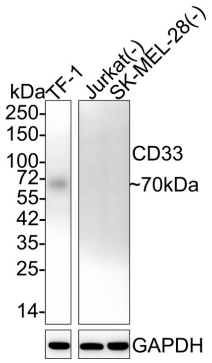

Fig1: Western blot analysis of CD33 on different lysates with Rabbit anti-CD33 antibody (HA721827) at 1/2,000 dilution.

Lane 1: TF-1 cell lysate
Lane 2: Jurkat cell lysate (negative)
Lane 3: SK-MEL-28 cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 40 kDa
Observed band size: 70 kDa

Exposure time: 3 minutes;

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 (HA721827) at 1/2,000 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.

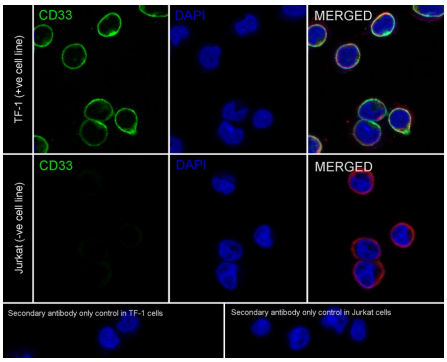

Fig2: Immunocytochemistry analysis of TF-1 (positive) and Jurkat (negative) labeling CD33 with Rabbit anti-CD33 antibody (HA721827) at 1/500 dilution.

Cells were fixed in 100% precooled methanol 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-CD33 antibody (HA721827) 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 (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.

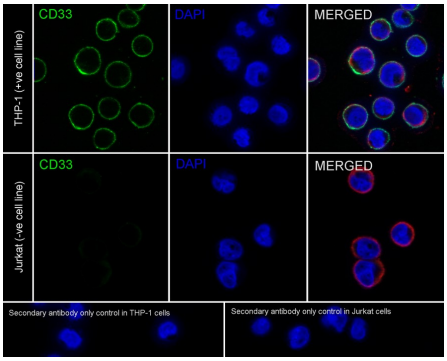

Fig3: Immunocytochemistry analysis of THP-1 (positive) and Jurkat (negative) labeling CD33 with Rabbit anti-CD33 antibody (HA721827) at 1/500 dilution.

Cells were fixed in 100% precooled methanol 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-CD33 antibody (HA721827) 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 (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.

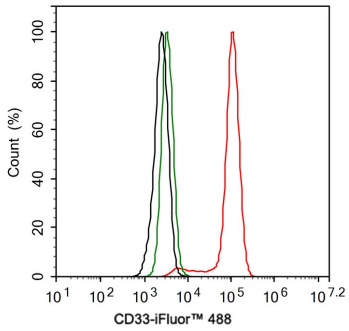

Fig4: Flow cytometric analysis of TF-1 cells labeling CD33.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (HA721827, 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).

Background References

1. Albinger N et al. Primary CD33-targeting CAR-NK cells for the treatment of acute myeloid leukemia. Blood Cancer J. 2022 Apr
2. Willier S et al. CLEC12A and CD33 coexpression as a preferential target for pediatric AML combinatorial immunotherapy. Blood. 2021 Feb