

Anti-CD134 Antibody [PSH10-14]

HA723183



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 29 kDa
Clone number:	PSH10-14

Description: Tumor necrosis factor receptor superfamily, member 4 (TNFRSF4), also known as CD134 and OX40 receptor, is a member of the TNFR-superfamily of receptors which is not constitutively expressed on resting naïve T cells, unlike CD28. OX40 is a secondary co-stimulatory immune checkpoint molecule, expressed after 24 to 72 hours following activation; its ligand, OX40L, is also not expressed on resting antigen presenting cells, but is following their activation. Expression of OX40 is dependent on full activation of the T cell; without CD28, expression of OX40 is delayed and of fourfold lower levels.

Immunogen: Recombinant protein within human CD134 aa 1-214.

Positive control: HUT 102 cell lysate, HUT 102.

Subcellular location: Membrane.

Database links: SwissProt: P43489 Human

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:500
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°C. Store at +4°C short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20°C long term.

Purity: Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

Technical:0086-571-89986345

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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

Fig1: Western blot analysis of CD134 on different lysates with Rabbit anti-CD134 antibody (HA723183) at 1/2,000 dilution.

Lane 1: HUT 102 cell lysate

Lane 2: Jurkat cell lysate (negative)

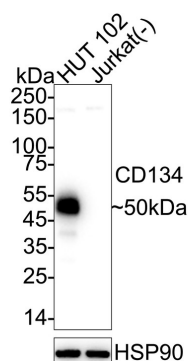
Lysates/proteins at 20 µg/Lane.

Predicted band size: 29 kDa

Observed band size: 50 kDa

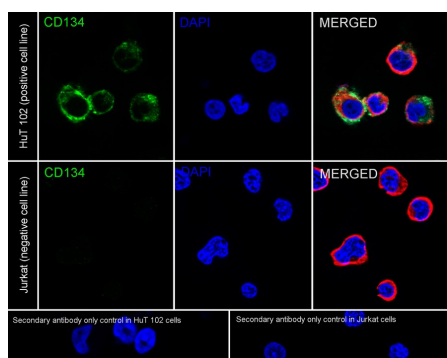
Exposure time: 6 seconds; ECL: K1801;

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 (HA723183) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C 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 HUT 102 (positive) and Jurkat (negative) labeling CD134 with Rabbit anti-CD134 antibody (HA723183) 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-CD134 antibody (HA723183) at 1/500 dilution in 1% BSA in PBST overnight at 4 °C. 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°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

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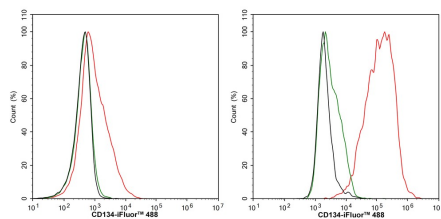


Fig3: Flow cytometric analysis of Jurkat (left, negative) and HUT 102 (right, positive) cells labeling CD134.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA723183, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4°C 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°C. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

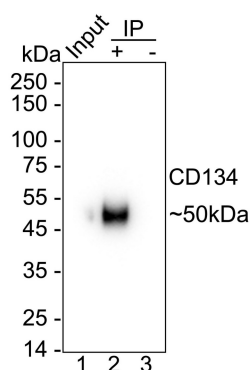


Fig4: CD134 was immunoprecipitated from 0.2 mg HUT 102 cell lysate with HA723183 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA723183 at 1/1,000 dilution. Mouse Anti-Rabbit IgG kappa light chain secondary antibody (M1208-2) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HUT 102 cell lysate (input)

Lane 2: HA723183 IP in HUT 102 cell lysate

Lane 3: Rabbit IgG instead of HA723183 in HUT 102 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST

Exposure time: 2 seconds; ECL: K1801

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

Background References

1. Kwiatek M et al. OX40 (CD134) Expression on T Regulatory Cells Is Related to Serious Hypertensive Disorders in Pregnancy. J Cardiovasc Dev Dis. 2023 Oct
2. Ansari AW et al. Azithromycin downregulates ICOS (CD278) and OX40 (CD134) expression and mTOR activity of TCR-activated T cells to inhibit proliferation. Int Immunopharmacol. 2023 Nov

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