Anti-CD276 Antibody [PSH02-46]

HA721823

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 57 kDa
Clone number:	PSH02-46

Description: May participate in the regulation of T-cell-mediated immune response. May play a protective role in tumor cells by inhibiting natural-killer mediated cell lysis as well as a role of marker for detection of neuroblastoma cells. May be involved in the development of acute and chronic transplant rejection and in the regulation of lymphocytic activity at mucosal surfaces. Could also play a key role in providing the placenta and fetus with a suitable immunological environment throughout pregnancy. Both isoform 1 and isoform 2 appear to be redundant in their ability to modulate CD4 T-cell responses. Isoform 2 is shown to enhance the induction of cytotoxic T-cells and selectively stimulates interferon gamma production in the presence of T-cell receptor signaling.

Immunogen: Full length recombinant protein within human CD276.

Positive control: HeLa cell lysate, MCF7 cell lysate, HEK-293 cell lysate, LoVo cell lysate, U-2 OS cell lysate, LNCaP cell lysate, SH-SY5Y cell lysate, THP-1 cell lysate, HCT 116 cell lysate, THP-1, MCF7.

Subcellular location: Membrane.

Database links: SwissProt: Q5ZPR3 Human

Recommended Dilutions:

WB	1:2,000-5,000
IF-Cell	1:100
FC	1:1,000

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

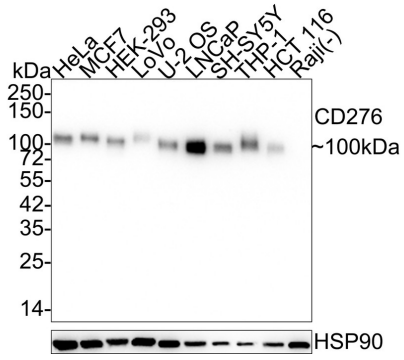

Fig1: Western blot analysis of CD276 on different lysates with Rabbit anti-CD276 antibody (HA721823) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: MCF7 cell lysate
Lane 3: HEK-293 cell lysate
Lane 4: LoVo cell lysate
Lane 5: U-2 OS cell lysate
Lane 6: LNCaP cell lysate
Lane 7: SH-SY5Y cell lysate
Lane 8: THP-1 cell lysate
Lane 9: HCT 116 cell lysate
Lane 10: Raji cell lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 57 kDa
Observed band size: 100 kDa

Exposure time: 42 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 (HA721823) 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.

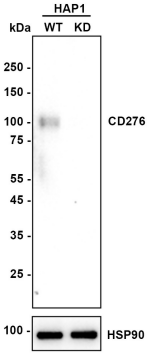

Fig2: Western blot analysis of CD276 on different lysates with Rabbit anti-CD276 antibody (HA721823) at 1/5,000 dilution.

Lane 1: HAP1-parental cell lysate
Lane 2: HAP1-CD276 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 57 kDa
Observed band size: 100 kDa

Exposure time: 180 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 (HA721823) at 1/5,000 dilution was used in K1803 at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody

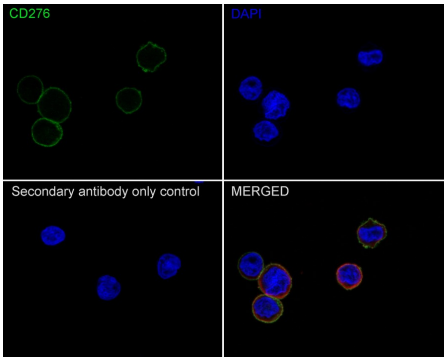

Fig3: Immunocytochemistry analysis of THP-1 cells labeling CD276 with Rabbit anti-CD276 antibody (HA721823) at 1/100 dilution.

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-CD276 antibody (HA721823) 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.

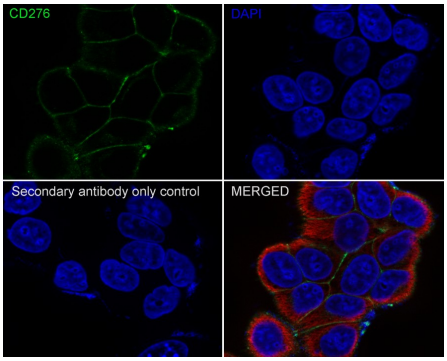

Fig4: Immunocytochemistry analysis of MCF7 cells labeling CD276 with Rabbit anti-CD276 antibody (HA721823) at 1/100 dilution.

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-CD276 antibody (HA721823) 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.

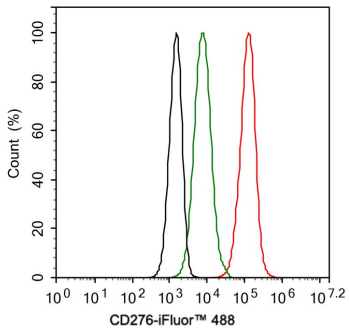

Fig5: Flow cytometric analysis of THP-1 cells labeling CD276.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (HA721823, 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. Chapoval A.I et al. B7-H3: a costimulatory molecule for T cell activation and IFN-gamma production. Nat Immunol 2:269-274 (2001).
2. Steinberger P et al. Molecular characterization of human 4Ig-B7-H3, a member of the B7 family with four Ig-like domains. J Immunol 172:2352-2359 (2004).