

Anti-CD40 Antibody [PSH05-90] - BSA and Azide free

HA751037



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Cynomolgus monkey, Pig
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 31 kDa
Clone number:	PSH05-90

Description: This gene is a member of the TNF-receptor superfamily. The encoded protein is a receptor on antigen-presenting cells of the immune system and is essential for mediating a broad variety of immune and inflammatory responses including T cell-dependent immunoglobulin class switching, memory B cell development, and germinal center formation. AT-hook transcription factor AKNA is reported to coordinately regulate the expression of this receptor and its ligand, which may be important for homotypic cell interactions. Adaptor protein TNFR2 interacts with this receptor and serves as a mediator of the signal transduction. The interaction of this receptor and its ligand is found to be necessary for amyloid-beta-induced microglial activation, and thus is thought to be an early event in Alzheimer disease pathogenesis. Mutations affecting this gene are the cause of autosomal recessive hyper-IgM immunodeficiency type 3 (HIGM3). Multiple alternatively spliced transcript variants of this gene encoding distinct isoforms have been reported.

Immunogen: Synthetic peptide within Human CD40 aa 228-277 / 277.

Positive control: Raji cell lysate, U-2 OS cell lysate, Ramos cell lysate, Daudi cell lysate, Raji.

Subcellular location: Cell membrane. Membrane. Secreted.

Database links: SwissProt: P25942 Human

Recommended Dilutions:

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

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

Technical:0086-571-89986345

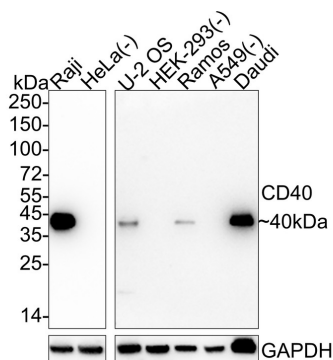
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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

Fig1: Western blot analysis of CD40 on different lysates with Rabbit anti-CD40 antibody (HA751037) at 1/1,000 dilution.



Lane 1: Raji cell lysate

Lane 2: HeLa cell lysate (negative)

Lane 3: U-2 OS cell lysate

Lane 4: HEK-293 cell lysate (negative)

Lane 5: Ramos cell lysate

Lane 6: A549 cell lysate (negative)

Lane 7: Daudi cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 31 kDa

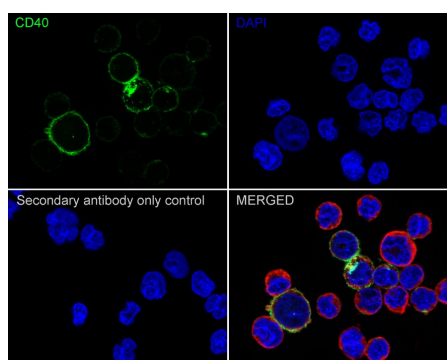
Observed band size: 40 kDa

Exposure time: Lane 1-2: 1 minute; Lane 3-7: 3 minutes; 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 (HA751037) at 1/1,000 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 Raji cells labeling CD40 with Rabbit anti-CD40 antibody (HA751037) 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-CD40 antibody (HA751037) at 1/100 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 (M1305-2, 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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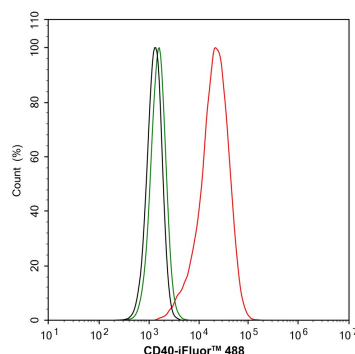


Fig3: Flow cytometric analysis of Raji cells labeling CD40.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751037, 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).

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

Background References

1. Remer M. et. al. The Use of Anti-CD40 mAb in Cancer. *Curr Top Microbiol Immunol*. 2017;405:165-207.
2. Zhang B. et. al. The CD40/CD40L system: a new therapeutic target for disease. *Immunol Lett*. 2013 Jun;153(1-2):58-61.

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