Anti-CD40 Antibody [PSH10-01] - BSA and Azide free

HA751331

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Cynomolgus monkey, Pig
Applications:	IHC-P, IHC-Fr, IF-Cell, FC, IF-Tissue
Molecular Wt:	Predicted band size: 31 kDa
Clone number:	PSH10-01

Description: Resting B cells can be activated and clonally expanded into antibody-producing cells in response to a combination of cell contact and soluble signals provided by primed helper T (Th) cells. A receptor ligand pair central to the trans-mission of this signal is CD40, expressed on the surface of B cells, together with CD40L, expressed on activated T cells. In the presence of such stimulus, IL-4 and IL-13 are capable of triggering immunoglobulin class switching and secretion of IgE. B cells are sensitive to these cytokines only subsequent to CD40/CD40L-driven DNA synthesis. A downstream mediator of the CD40 signaling pathway, designated CRAF, is a member of an expanding family of proteins that contain a conserved cysteine- and histidine-rich RING finger motif.

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

Positive control: Human spleen tissue, mouse spleen tissue, rat spleen tissue, Raji, A20, PC-12.

Subcellular location: Cell membrane. Secreted.

Database links: SwissProt: P25942 Human | P27512 Mouse
Entrez Gene: 171369 Rat

Recommended Dilutions:

IHC-P	1:1,000
IHC-Fr	1:1,000
IF-Cell	1:50-1:500
FC	1:1,000
IF-Tissue	1:500

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.

Images

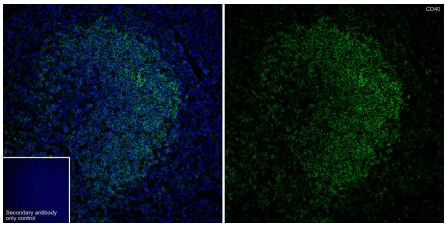

Fig1: Application: IHC-Fr

Species: Mouse

Site: Spleen

Sample: Frozen section

Antibody concentration: 1:500

Antigen retrieval: Not required

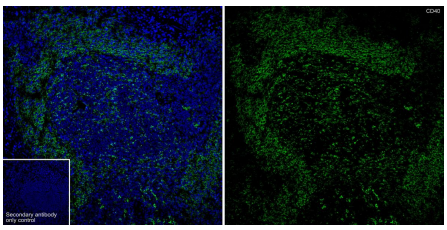

Fig2: Application: IHC-Fr

Species: Rat

Site: Spleen

Sample: Frozen section

Antibody concentration: 1:500

Antigen retrieval: Not required

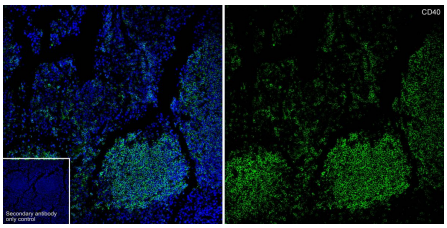

Fig3: Application: IF-Tissue

Species: Mouse

Site: spleen

Sample: Paraffin-embedded section

Antibody concentration: 1/500

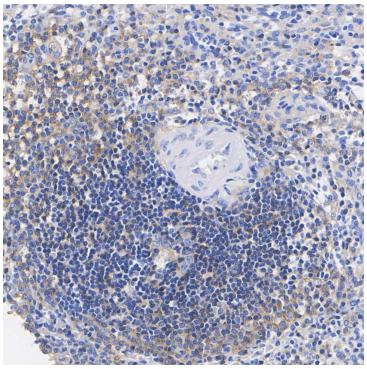

Fig4: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-CD40 antibody (HA751331) at 1/1,000 dilution.

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 (HA751331) at 1/1,000 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.

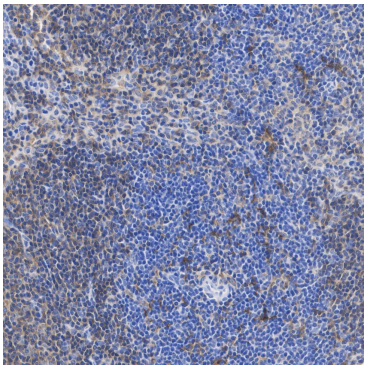

Fig5: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-CD40 antibody (HA751331) at 1/1,000 dilution.

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 (HA751331) at 1/1,000 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.

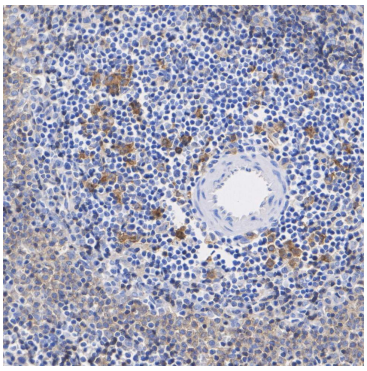

Fig6: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-CD40 antibody (HA751331) at 1/1,000 dilution.

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 (HA751331) at 1/1,000 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.

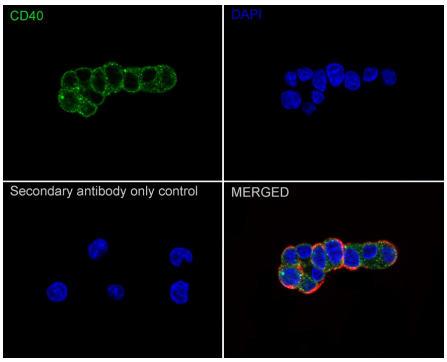

Fig7: Immunocytochemistry analysis of Raji cells labeling CD40 with Rabbit anti-CD40 antibody (HA751331) at 1/500 dilution.

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-CD40 antibody (HA751331) 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 (HA601187, 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.

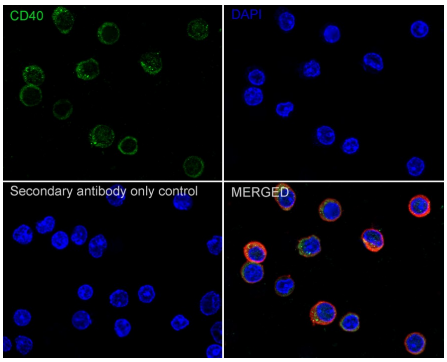

Fig8: Immunocytochemistry analysis of A20 cells labeling CD40 with Rabbit anti-CD40 antibody (HA751331) at 1/50 dilution.

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-CD40 antibody (HA751331) at 1/50 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 (HA601187, 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.

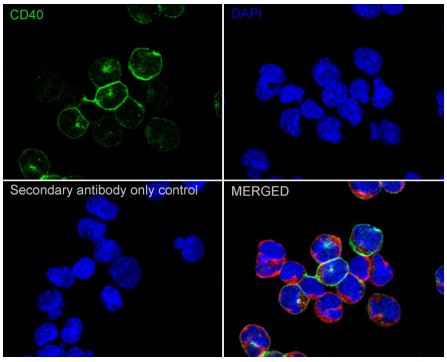

Fig9: Immunocytochemistry analysis of PC-12 cells labeling CD40 with Rabbit anti-CD40 antibody (HA751331) at 1/100 dilution.

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-CD40 antibody (HA751331) 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 (HA601187, 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.

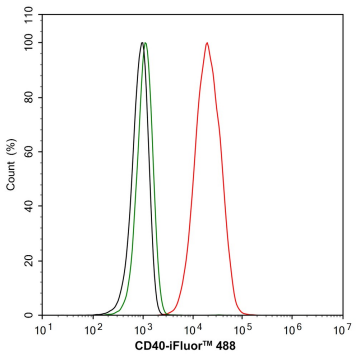

Fig10: Flow cytometric analysis of A20 cells labeling CD40.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751331, 1/1,000) (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. Contin C et al. Membrane-anchored CD40 is processed by the tumor necrosis factor-α-converting enzyme. Implications for CD40 sig- naling. J Biol Chem 278: 32801-32809(2013).
2. Li G et al. 2013. Human genetics in rheumatoid arthritis guides a high-throughput drug screen of the CD40 signaling pathway. PLoS Genet 9: e1003487(2013).