Anti-CD3 Antibody [PSH19-70] - BSA and Azide free

HA751725

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell, FC, mIHC
Molecular Wt:	Predicted band size: 23 kDa
Clone number:	PSH19-70

Description: The CD3 protein is a T-cell marker, a complex of four structurally distinct membrane glycoprotein isoforms, 20-50 kDa, comprising extracellular, transmembrane and intracellular domains. The CD3 complex is responsible for mediating signal transduction to the internal environment upon antigenic recognition by TCR, causing T-cell proliferation and release of cytokines. Except for a weak expression in Purkinje cells (with some of the Abs) and activated NK-cells, CD3 is found only in T-cells. CD3 appear in the cytoplasm of prothymocytes, and on the surface of about 95% of thymocytes, while cytoplasmic CD3 is lost as the cells differentiate into medullary thymocytes. In therapy resistant celiac disease, a shift from membranous to cytoplasmic CD3 expression is seen (together with loss of CD8). In malignant lymphomas, CD3 is a pan-T-cell lineage-restricted antigen, detected in 80-97% of the T-cell lymphomas. Mature T-cell lymphomas including cases of mycosis fungoides, peripheral T-cell lymphoma and anaplastic large cell lymphoma may aberrantly lose CD3. NK-cell lymphomas can show a cytoplasmic reaction. Reed-Sternberg cells may show a globular paranuclear reaction. CD3 is an important marker in the classification of malignant lymphomas and lymphoid leukaemias. Also the marker is useful for the identification of T-cells in, e.g., celiac disease, lymphocytic colitis and colorectal carcinomas associated with loss of a mismatch repair protein.

Positive control:	MOLT-4 cell lysate, Jurkat cell lysate, mouse spleen tissue, rat spleen tissue, MOLT-4, mouse spleen.
Subcellular location:	Cell membrane.
Database links:	SwissProt: P07766 Human
Recommended Dilutions:	
WB	1:2,000
IHC-P	1:8,000
IF-Cell	1:500
FC	1:1,000
mIHC	1:200-1:1,000
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.

Images

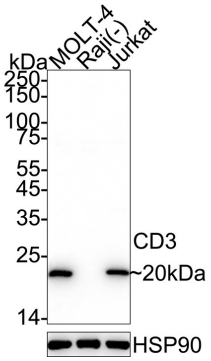

Fig1: Western blot analysis of CD3 on different lysates with Rabbit anti-CD3 antibody (HA751725) at 1/2,000 dilution.

Lane 1: MOLT-4 cell lysate
Lane 2: Raji cell lysate (negative)
Lane 3: Jurkat cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 23 kDa
Observed band size: 20 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 (HA751725) at 1/2,000 dilution was used in primary antibody dilution (K1803) at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

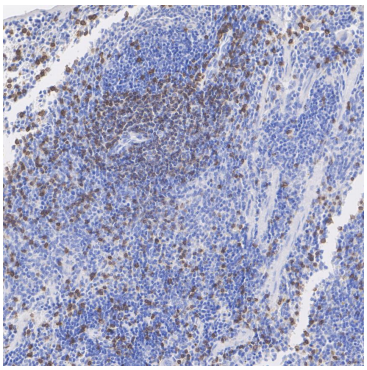

Fig2: Immunohistochemical analysis of paraffin-embedded mouse spleen tissue with Rabbit anti-CD3 antibody (HA751725) at 1/8,000 dilution.

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 (HA751725) at 1/8,000 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.

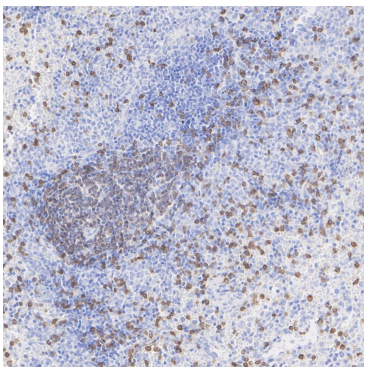

Fig3: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-CD3 antibody (HA751725) at 1/8,000 dilution.

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 (HA751725) at 1/8,000 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.

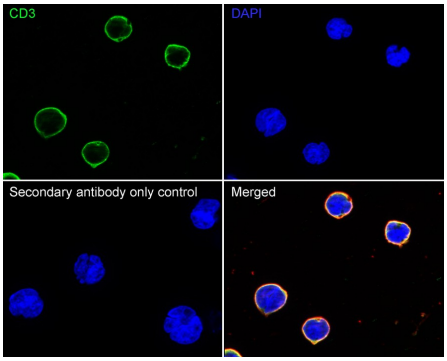

Fig4: Immunocytochemistry analysis of MOLT-4 cells labeling CD3 with Rabbit anti-CD3 antibody (HA751725) at 1/500 dilution.

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-CD3 antibody (HA751725) 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.

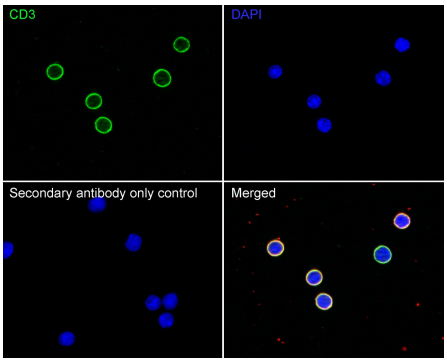

Fig5: Immunocytochemistry analysis of mouse spleen cells labeling CD3 with Rabbit anti-CD3 antibody (HA751725) at 1/500 dilution.

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-CD3 antibody (HA751725) 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.

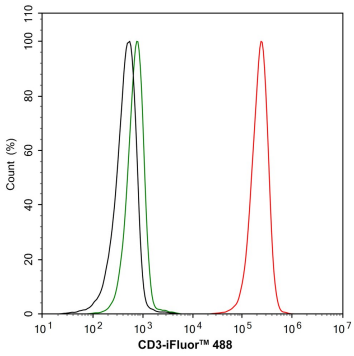

Fig6: Flow cytometric analysis of MOLT-4 cells labeling CD3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751725, 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. Erman B. et. al. Biallelic Form of a Known CD3E Mutation in a Patient with Severe Combined Immunodeficiency. J Clin Immunol. 2020 Apr
2. Chen Q. et. al. CD3(+)CD20(+) T cells and their roles in human diseases. Hum Immunol. 2019 Mar