Anti-CD3 Antibody [PSH22-52] - BSA and Azide free

HA751865

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-Fr, IHC-P, IF-Tissue, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 23 kDa
Clone number:	PSH22-52

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.

Immunogen:	Recombinant protein within mouse CD3E aa 1-207.
Positive control:	MOLT-4 cell lysate, Jurkat cell lysate, Mouse thymus tissue lysate, Mouse spleen tissue lysate, Rat spleen tissue lysate, rat colon tissue, rat spleen tissue, Jurkat, MOLT-4, mouse splenocytes.
Subcellular location:	Cell membrane.
Database links:	SwissProt: P07766 Human | P22646 Mouse Entrez Gene: 315609 Rat

Recommended Dilutions:

WB	1:5,000-1:20,000
IHC-Fr	1:500
IHC-P	1:5,000
IF-Tissue	1:500
IF-Cell	1:200-1:2,500
FC	1:1,000
IP	1-2μg/sample

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

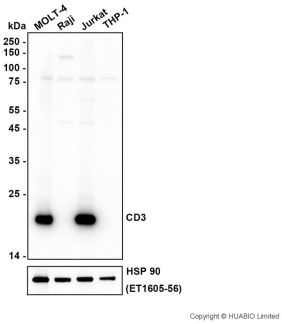

Fig1: Western blot analysis of CD3 on different lysates with Rabbit anti-CD3 antibody (HA751865) at 1/20,000 dilution.

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

Lysates/proteins at 10 µg/Lane.

Predicted band size: 23 kDa
Observed band size: 20 kDa

Exposure time: 4 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 (HA751865) at 1/20,000 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.

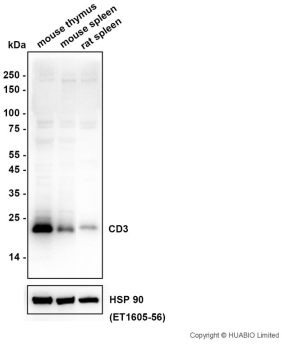

Fig2: Western blot analysis of CD3 on different lysates with Rabbit anti-CD3 antibody (HA751865) at 1/5,000 dilution.

Lane 1: Mouse thymus tissue lysate
Lane 2: Mouse spleen tissue lysate
Lane 3: Rat spleen tissue lysate

Lysates/proteins at 30 µg/Lane.

Predicted band size: 21 kDa
Observed band size: 21 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 (HA751865) at 1/5,000 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.

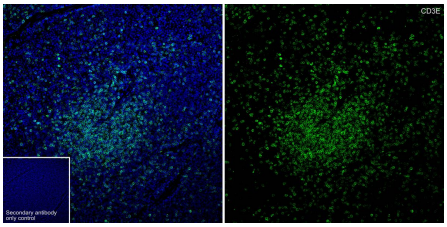

Fig3: Application: IHC-Fr

Species: Mouse

Site: spleen

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

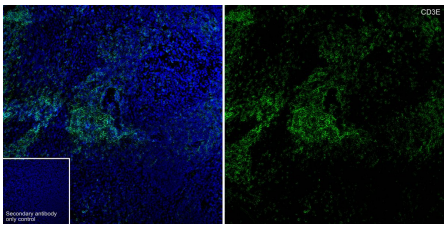

Fig4: Application: IHC-Fr

Species: Rat

Site: spleen

Sample: Frozen section

Antibody concentration: 1/500

Antigen retrieval: Not required

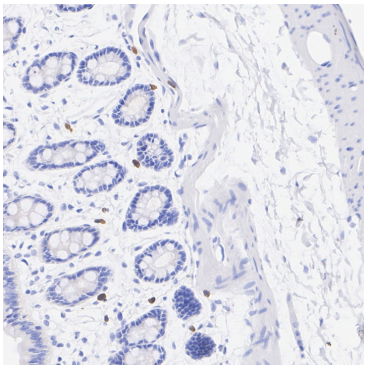

Fig5: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-CD3 antibody (HA751865) at 1/5,000 dilution.

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 (HA751865) at 1/5,000 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.

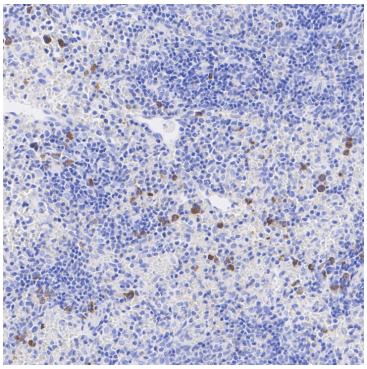

Fig6: Immunohistochemical analysis of paraffin-embedded rat spleen tissue with Rabbit anti-CD3 antibody (HA751865) at 1/5,000 dilution.

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 (HA751865) at 1/5,000 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.

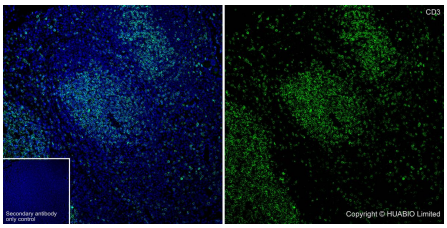

Fig7: Application: IF-Tissue

Species: Mouse

Site: spleen

Sample: Paraffin-embedded section

Antibody concentration: 1/500

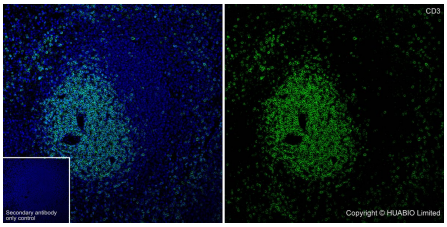

Fig8: Application: IF-Tissue

Species: Rat

Site: spleen

Sample: Paraffin-embedded section

Antibody concentration: 1/500

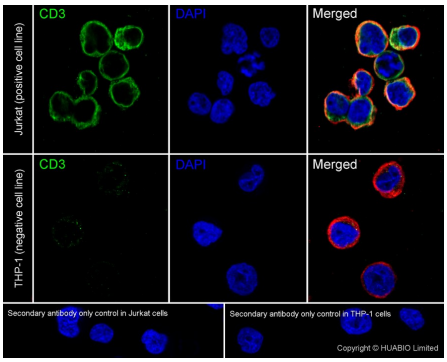

Fig9: Immunocytochemistry analysis of Jurkat (positive) and THP-1 (negative) labeling CD3 with Rabbit anti-CD3 antibody (HA751865) at 1/500 dilution.

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 (HA751865) 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.

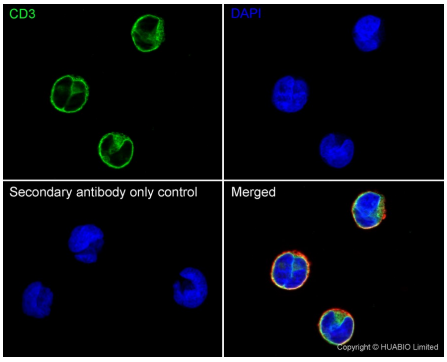

Fig10: Immunocytochemistry analysis of MOLT-4 cells labeling CD3 with Rabbit anti-CD3 antibody (HA751865) at 1/2,500 dilution.

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 (HA751865) at 1/2,500 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.

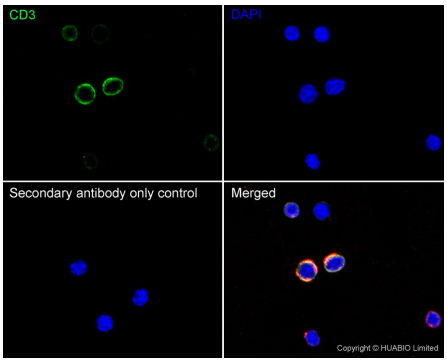

Fig11: Immunocytochemistry analysis of mouse splenocytes labeling CD3 with Rabbit anti-CD3 antibody (HA751865) at 1/200 dilution.

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 (HA751865) at 1/200 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.

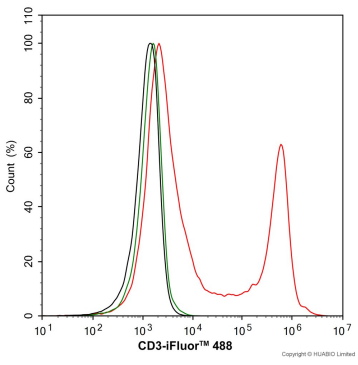

Fig12: Flow cytometric analysis of MOLT-4 cells labeling CD3.

Cells were washed twice with cold PBS and resuspend. Then stained with the primary antibody (HA751865, 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).

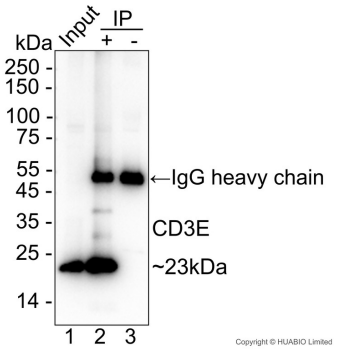

Fig13: CD3 was immunoprecipitated from 0.2 mg MOLT-4 cell lysate with HA751865 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751865 at 1/10,000 dilution. Alpaca anti-Rabbit IgG Fc secondary antibody (HA1031) at 1/50,000 dilution was used for 1 hour at room temperature.

Lane 1: MOLT-4 cell lysate (input)
Lane 2: HA751865 IP in MOLT-4 cell lysate
Lane 3: Rabbit IgG instead of HA751865 in MOLT-4 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 7 seconds; ECL: K1801

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