Anti-ZAP70 Antibody [JU08-39]

ET1706-42

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IHC-P, FC, IP
Molecular Wt:	Predicted band size: 70 kDa
Clone number:	JU08-39

Description: ZAP-70 (Zeta-chain-associated protein kinase 70) is a protein normally expressed near the surface membrane of lymphocytes (T cells, natural killer cells, and a subset of B cells). It is most prominently known to be recruited upon antigen binding to the T cell receptor (TCR), and it plays a critical role in T cell signaling. ZAP-70 was initially discovered in TCR-stimulated Jurkat cells, an immortal line of human T lymphocytes, in 1991. Its molecular weight is 70 kDa, and it is a member of the protein-tyrosine kinase family and is a close homolog of SYK. SYK and ZAP70 share a common evolutionary origin and split from a common ancestor in the jawed vertebrates. The importance of ZAP-70 in T cell activation was determined when comparing ZAP-70 expression in patients with SCID (severe combined immunodeficiency). ZAP-70 deficient individuals were found to have no functioning T cells in their peripheral blood, suggesting that ZAP-70 is a critical component of T cell activation and development. ZAP-70 expression in B cells is correlated with the development of chronic lymphocytic leukemia (CLL).

Immunogen: Synthetic peptide within Human ZAP70 aa 570-619 / 619.

Positive control: Jurkat cell lysate, MOLT-4 cell lysate, HUT 102 cell lysate, human lymph node tissue, human spleen tissue, LOVO, SH-SY-5Y, Jurkat.

Subcellular location: Cytoplasm. Cell membrane.

Database links: SwissProt: P43403 Human

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50-1:200
IHC-P	1:200
FC	1:50-1:100
IP	1:10-1:50

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

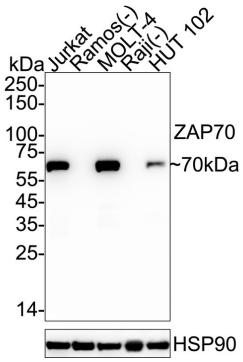

Fig1: Western blot analysis of ZAP70 on different lysates with Rabbit anti-ZAP70 antibody (ET1706-42) at 1/2,000 dilution.

Lane 1: Jurkat cell lysate (20 µg/Lane)
Lane 2: Ramos cell lysate (negative) (20 µg/Lane)
Lane 3: MOLT-4 cell lysate (20 µg/Lane)
Lane 4: Raji cell lysate (negative) (20 µg/Lane)
Lane 5: HUT 102 cell lysate (20 µg/Lane)

Predicted band size: 70 kDa
Observed band size: 70 kDa

Exposure time: 40 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 (ET1706-42) 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.

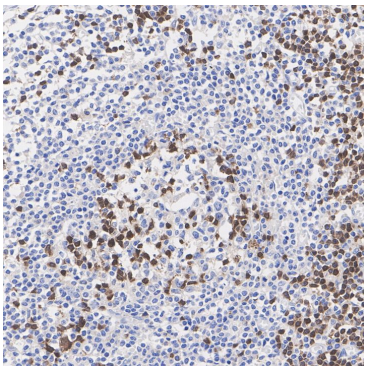

Fig2: Immunohistochemical analysis of paraffin-embedded human lymph node tissue with Rabbit anti-ZAP70 antibody (ET1706-42) at 1/200 dilution.

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 (ET1706-42) at 1/200 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.

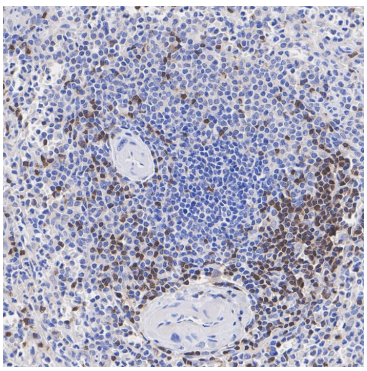

Fig3: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-ZAP70 antibody (ET1706-42) at 1/200 dilution.

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 (ET1706-42) at 1/200 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.

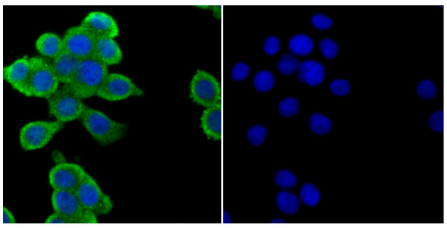

Fig4: Immunocytochemistry analysis of LOVO cells labeling ZAP70 with Rabbit anti-ZAP70 antibody (ET1706-42) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-ZAP70 antibody (ET1706-42) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

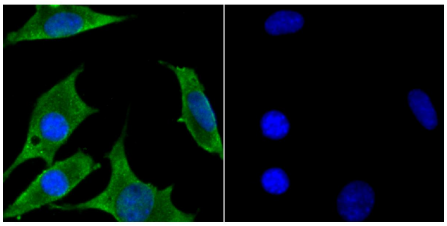

Fig5: Immunocytochemistry analysis of SH-SY-5Y cells labeling ZAP70 with Rabbit anti-ZAP70 antibody (ET1706-42) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-ZAP70 antibody (ET1706-42) at 1/50 dilution in 2% negative goat serum overnight at 4 ℃. Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. Nuclear DNA was labelled in blue with DAPI.

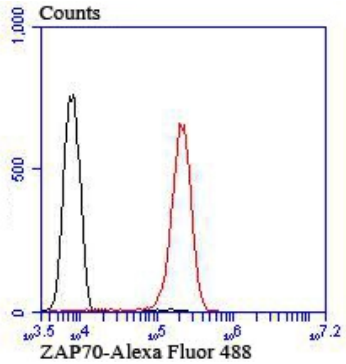

Fig6: Flow cytometric analysis of ZAP70 was done on Jurkat cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1706-42, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.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. Ashouri JF et al. ZAP70, too little, too much can lead to autoimmunity. Immunol Rev. 2022 May
2. Leveille E et al. SYK and ZAP70 kinases in autoimmunity and lymphoid malignancies. Cell Signal. 2022 Jun