Anti-ZAP70 Antibody [A1B5-R]

HA601218

Product Type:	Recombinant Mouse monoclonal IgG1, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P
Molecular Wt:	Predicted band size: 70 kDa
Clone number:	A1B5-R

Description: Tyrosine kinase that plays an essential role in regulation of the adaptive immune response. Regulates motility, adhesion and cytokine expression of mature T-cells, as well as thymocyte development. Contributes also to the development and activation of primary B-lymphocytes. When antigen presenting cells (APC) activate T-cell receptor (TCR), a serie of phosphorylations lead to the recruitment of ZAP70 to the doubly phosphorylated TCR component CD247/CD3Z through ITAM motif at the plasma membrane. This recruitment serves to localization to the stimulated TCR and to relieve its autoinhibited conformation. Release of ZAP70 active conformation is further stabilized by phosphorylation mediated by LCK. In turn, a large number of signaling molecules are recruited and ultimately lead to lymphokine production, T-cell proliferation and differentiation. Furthermore, ZAP70 controls cytoskeleton modifications, adhesion and mobility of T-lymphocytes, thus ensuring correct delivery of effectors to the APC. ZAP70 is also required for TCR-CD247/CD3Z internalization and degradation through interaction with the E3 ubiquitin-protein ligase CBL and adapter proteins SLA and SLA2. Thus, ZAP70 regulates both T-cell activation switch on and switch off by modulating TCR expression at the T-cell surface.

Immunogen: Recombinant protein within human ZAP70 aa 250-480.

Positive control: Jurkat cell lysate, mouse spleen tissue lysate, mouse thymus tissue lysate, rat spleen tissue lysate, rat thymus tissue lysate, human tonsil tissue.

Subcellular location: Cell membrane, cytoplasm.

Database links: SwissProt: P43403 Human | P43404 Mouse
Entrez Gene: 301348 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:2,000

Storage Buffer: PBS (pH7.4), 0.1% 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

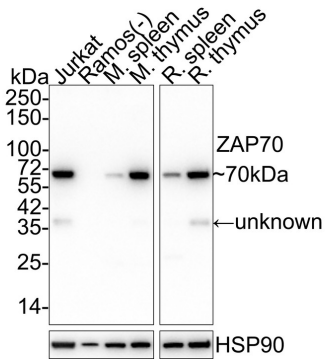

Fig1: Western blot analysis of ZAP70 on different lysates with Mouse anti-ZAP70 antibody (HA601218) at 1/1,000 dilution.

Lane 1: Jurkat cell lysate
Lane 2: Ramos cell lysate (negative)
Lane 3: Mouse spleen tissue lysate
Lane 4: Mouse thymus tissue lysate
Lane 5: Rat spleen tissue lysate
Lane 6: Rat thymus tissue lysate

Lysates/proteins at 20 µg/Lane.

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

Exposure time: 1 minute 21 seconds;

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 (HA601218) at 1/1,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Mouse IgG - HRP Secondary Antibody (HA1006) at 1/50,000 dilution was used for 1 hour at room temperature.

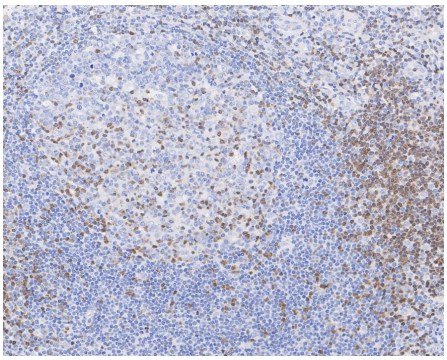

Fig2: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Mouse anti-ZAP70 antibody (HA601218) at 1/2,000 dilution.

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 (HA601218) at 1/2,000 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.

Background References

1. Fischer A. et. al. ZAP70: a master regulator of adaptive immunity. Semin. Immunopathol. 32:107-116(2010).
2. Hu H. et. al. Otud7b facilitates T cell activation and inflammatory responses by regulating Zap70 ubiquitination. J. Exp. Med. 213:399-414(2016)

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