10320 Camino Santa Fe, Suite G
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Tel: 858.875.1900 Fax: 858.875.1999

# Anti-ZAP70 Antibody Picoband™ (monoclonal, 9D5)

**Catalog # ABO14951**

## Specification

### Anti-ZAP70 Antibody Picoband™ (monoclonal, 9D5) - Product Information

| | |
|---|---|
| Application | **WB, FC** |
| Primary Accession | P43403 |
| Host | **Mouse** |
| Isotype | **Mouse IgG2b** |
| Reactivity | **Rat, Human, Mouse** |
| Clonality | **Monoclonal** |
| Format | **Lyophilized** |

**Description**
Anti-ZAP70 Antibody Picoband™ (monoclonal, 9D5) . Tested in Flow Cytometry, WB applications. This antibody reacts with Human, Mouse, Rat.

**Reconstitution**
Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

### Anti-ZAP70 Antibody Picoband™ (monoclonal, 9D5) - Additional Information

**Gene ID** 7535

**Other Names**
Tyrosine-protein kinase ZAP-70, 2.7.10.2, 70 kDa zeta-chain associated protein, Syk-related tyrosine kinase, ZAP70, SRK

**Calculated MW**
72 kDa KDa

**Application Details**
Western blot, 0.1-0.5 µg/ml, Human, Mouse, Rat<br> Flow Cytometry, 1-3 µg/1x10^6 cells, Human<br>

**Subcellular Localization**
Cell membrane. Cytoplasm. Membrane.

**Tissue Specificity**
Expressed in T- and natural killer cells. Also present in early thymocytes and pro/pre B-cells.

**Contents**
Each vial contains 4mg Trehalose, 0.9mg NaCl, 0.2mg Na<sub>2</sub>HPO<sub>4</sub>, 0.05mg NaN<sub>3</sub>.

**Immunogen**
A synthetic peptide corresponding to a sequence in the middle region of human ZAP70, different from the related mouse sequence by two amino acids.

**Purification**

Immunogen affinity purified.

**Cross Reactivity**
No cross-reactivity with other proteins.

| | |
|---|---|
| Storage | **Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.** |

## Anti-ZAP70 Antibody Picoband™ (monoclonal, 9D5) - Protein Information

**Name** ZAP70

**Synonyms** SRK

**Function**
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. Subsequently, ZAP70 phosphorylates at least 2 essential adapter proteins: LAT and LCP2. 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. During thymocyte development, ZAP70 promotes survival and cell-cycle progression of developing thymocytes before positive selection (when cells are still CD4/CD8 double negative). Additionally, ZAP70-dependent signaling pathway may also contribute to primary B-cells formation and activation through B-cell receptor (BCR).

**Cellular Location**
Cytoplasm. Cell membrane; Peripheral membrane protein. Note=In quiescent T-lymphocytes, it is cytoplasmic. Upon TCR activation, it is recruited at the plasma membrane by interacting with CD247/CD3Z. Colocalizes together with RHOH in the immunological synapse. RHOH is required for its proper localization to the cell membrane and cytoskeleton fractions in the thymocytes (By similarity).

**Tissue Location**
Expressed in T- and natural killer cells. Also present in early thymocytes and pro/pre B-cells

## Anti-ZAP70 Antibody Picoband™ (monoclonal, 9D5) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides

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- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

**Anti-ZAP70 Antibody Picoband™ (monoclonal, 9D5) - Images**

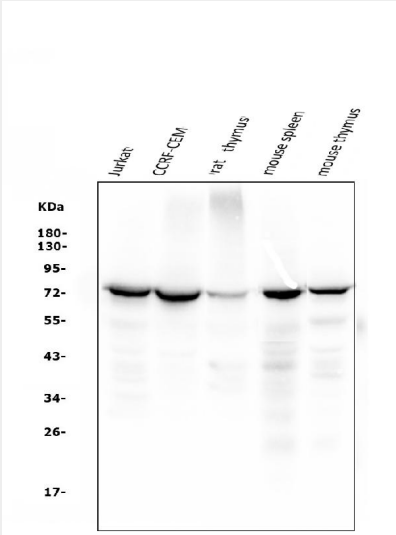


Figure 1. Western blot analysis of ZAP70 using anti-ZAP70 antibody (M00754-5).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: human Jurkat whole cell lysates;
Lane 2: human CCRF-CEM whole cell lysates;
Lane 3: rat thymus tissue lysates;
Lane 4: mouse spleen tissue lysates;
Lane 5: mouse thymus tissue lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-ZAP70 antigen affinity purified monoclonal antibody (Catalog # M00754-5) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for ZAP70 at approximately 72KD. The expected band size for ZAP70 is at 70KD.

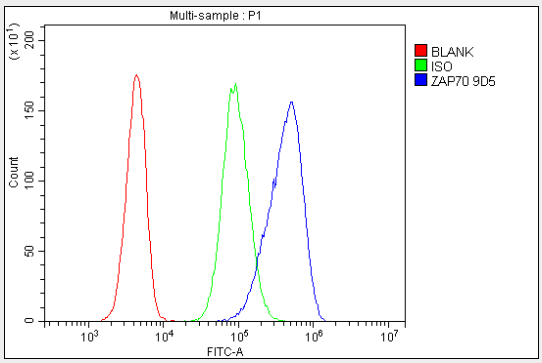

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Figure 2. Flow Cytometry analysis of 293T cells using anti-ZAP70 antibody (M00754-5).
Overlay histogram showing 293T cells stained with M00754-5 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-ZAP70 Antibody (M00754-5, 1 µg/1x$10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x$10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x$10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

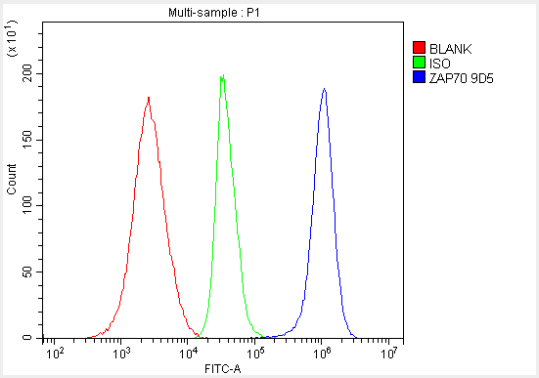


Figure 3. Flow Cytometry analysis of Jurkat cells using anti-ZAP70 antibody (M00754-5).
Overlay histogram showing Jurkat cells stained with M00754-5 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-ZAP70 Antibody (M00754-5, 1 µg/1x$10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x$10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x$10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

**Anti-ZAP70 Antibody Picoband™ (monoclonal, 9D5) - Background**

ZAP-70 (Zeta-chain-associated protein kinase 70) encodes an enzyme belonging to the protein tyrosine kinase family, and it plays a role in T-cell development and lymphocyte activation. This enzyme, which is phosphorylated on tyrosine residues upon T-cell antigen receptor (TCR) stimulation, functions in the initial step of TCR-mediated signal transduction in combination with the Src family kinases, Lck and Fyn. This enzyme is also essential for thymocyte development. Mutations in this gene cause selective T-cell defect, a severe combined immunodeficiency disease characterized by a selective absence of CD8-positive T-cells. Two transcript variants that encode different isoforms have been found for this gene.