abcam

ab247256

# Anti-ZAP70 antibody [E267] - BSA and Azide free

Recombinant | RabMAb

## Key facts

| | |
|---|---|
| **Isotype** | IgG |
| **Host species** | Rabbit |
| **Storage buffer** | pH: 7.2 - 7.4 Constituents: PBS |
| **Form** | Liquid |
| **Clonality** | Monoclonal |
| **Immunogen** | The exact immunogen used to generate this antibody is proprietary information. |
| **Carrier free** | Yes |
| **Clone number** | E267 |
| **Purification technique** | Affinity purification Protein A |
| **Specificity** | This antibody recognises Zap-70, a Syk-family protein tyrosine kinase. |

## Reactivity data

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt

### Tested

| | |
|---|---|
| **Species** | Human, Mouse |
| **Dilution info** | - |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | - |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

### Predicted

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | - |
| **Notes** | - |

## Target data

See full target information ZAP70

**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. Also contributes 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 phosphorylated TCR components CD3E and CD247/CD3Z through ITAM motif at the plasma membrane (PubMed:7509083). 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).

## Storage

| | |
|---|---|
| **Shipped at conditions** | Blue Ice |
| **Appropriate short-term storage conditions** | +4°C |

| | |
|---|---|
| **Appropriate long-term storage conditions** | +4°C |
| **Storage information** | Do Not Freeze |

## Notes

ab247256 is the carrier-free version of ab32410.

**Patented technology**

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

**What are the advantages of a recombinant monoclonal antibody?**

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility
- Improved sensitivity and specificity
- Long-term security of supply
- Animal-free batch production

For more information, read more on recombinant antibodies.

**Conjugation ready**

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

**Compatibility**

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

## Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

| | |
|---|---|
| **Activity summary** | ZAP70 also known as Zeta-chain-associated protein kinase 70 is a tyrosine kinase that plays an important role in T-cell receptor signaling. The protein has a molecular mass of approximately 70 kDa. ZAP70 is mainly expressed in T cells and natural killer (NK) cells. It gets activated upon T-cell receptor engagement initiating downstream signaling cascades essential for T-cell function and adaptive immune response. |
| **Biological function summary** | ZAP70 is instrumental in the signaling cascade that activates T cells in response to antigen recognition. It forms part of a larger signaling complex following the engagement of the T-cell receptor with antigens. This complex transmits critical activation signals leading to further cellular responses such as cytokine production |

| | |
|---|---|
| | and cell proliferation. ZAP70's function ensures appropriate immune responses and helps maintain immune system balance. |
| **Pathways** | ZAP70 plays an integral role in T-cell receptor signaling pathways. This protein interacts with other signaling molecules like Lck and LAT facilitating the transmission of activation signals within the cell. As part of the T-cell activation pathway ZAP70 helps to bridge receptor-ligand interactions with cellular responses enabling the immune cells to combat infections effectively. |
| **Associated diseases and disorders** | ZAP70 mutations or dysregulations can lead to severe immunodeficiencies or autoimmune diseases. For example ZAP70 deficiency results in severe combined immunodeficiency (SCID) which severely impairs immune function. Additionally aberrant ZAP70 activity has links to certain autoimmune disorders like rheumatoid arthritis where it influences abnormal immune signaling. Understanding the biochemical pathways that involve ZAP70 provides insight into managing these diseases and developing therapeutic strategies. |

## Product promise

**Tested**

We have tested this species and application combination and it works. It is covered by our product promise.

**Expected**

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

**Predicted**

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

**Not recommended**

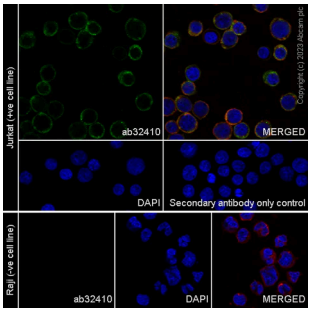
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

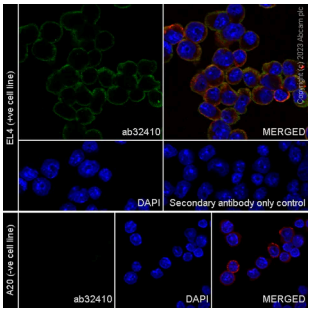
Full details and terms and conditions can be found here:
Terms & Conditions.

## 5 product images



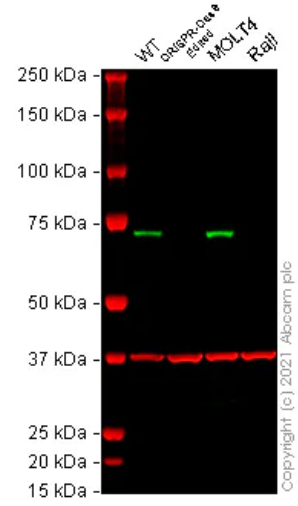

## Immunocytochemistry/ Immunofluorescence - Anti-ZAP70 antibody [E267] - BSA and Azide free (ab247256)

Immunocytochemistry/ Immunofluorescence analysis of Jurkat (human T cell leukemia T lymphocyte from peripheral blood) labeling ZAP70 with ab32410 at 1/100 dilution followed by ab150081 at 1/1000 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% TritonX-100. ab195889 at 1/200 was used as counterstain antibody. Nuclear counterstain was DAPI. Confocal image showing positive staining in Jurkat cell line (shown in green). The counterstain was observed with red. Nuclear DNA was labeled with DAPI (shown in blue). Negative control: Raji. Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8). This data was developed using ab32410, the same antibody clone in a different buffer formulation.




## Immunocytochemistry/ Immunofluorescence - Anti-ZAP70 antibody [E267] - BSA and Azide free (ab247256)

Immunocytochemistry/ Immunofluorescence analysis of EL4 (mouse lymphoma T lymphocyte) labeling ZAP70 with ab32410 at 1/100 dilution followed by ab150081 at 1/1000 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% TritonX-100. ab195889 at 1/200 was used as counterstain antibody. Nuclear counterstain was DAPI. Confocal image showing positive staining in El4 cell line (shown in green). The counterstain was observed with red. Nuclear DNA was labeled with DAPI (shown in blue). Negative control: A20. Image was taken with a confocal microscope(Leica-Microsystems, TCS SP8). This data was developed using ab32410, the same antibody clone in a different buffer formulation.




## Western blot - Anti-ZAP70 antibody [E267] - BSA and Azide free (ab247256)

ZAP70 Western blot staining using rabbit Anti-ZAP70 antibody

False colour image of Western blot: Anti-ZAP70 antibody [E267] staining at 1/500 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab32410 was shown to bind specifically to ZAP70. A band was observed at 70 kDa in wild-type Jurkat cell lysates with no signal observed at this size in ZAP70 CRISPR-Cas9 edited cell line ab273841 (CRISPR-Cas9 edited cell lysate ab273795). The band observed in the CRISPR-Cas9 edited lysate lane below 70 kDa is likely to represent a truncated form of ZAP70. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and ZAP70 CRISPR-Cas9 edited Jurkat cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.
All lanes:
Western blot - Anti-ZAP70 antibody [E267] (ab32410) at 1/500 dilution

Lane 1:
Wild-type Jurkat cell lysate at 20 µg

Lane 2:
ZAP70 CRISPR-Cas9 edited Jurkat cell lysate at 20 µg

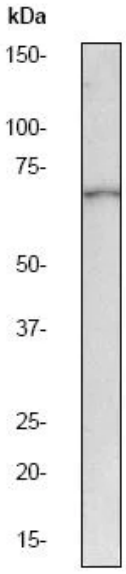
Lane 3:
MOLT-4 cell lysate at 20 µg

Lane 4:
Raji cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 69 kDa

Observed band size: 70 kDa




## Western blot - Anti-ZAP70 antibody [E267] - BSA and Azide free (ab247256)

ZAP70 Western blot staining of Jurkat cell lysates using rabbit Anti-ZAP70 antibody

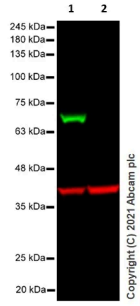
This data was developed using ab32410, the same antibody clone in a different buffer formulation. Western blot analysis on Jurkat Cell lysate.

All lanes:
Western blot - Anti-ZAP70 antibody [E267] (ab32410) at 1/500 dilution

All lanes:
Jurkat cell lysates

Predicted band size: 69 kDa

Observed band size: 70 kDa




## Western blot - Anti-ZAP70 antibody [E267] - BSA and Azide free (ab247256)

ZAP70 Western blot staining using rabbit Anti-ZAP70 antibody

Anti-GAPDH antibody, ab8245 (1/20000) was used as a primary antibody for the loading control and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed, ab216776 (1/10000) was used as a loading control secondary antibody.
Lanes 1-2: Merged signal (red and green). Green – ab32410 observed at 70 kDa. Red - loading control ab8245 observed at 36 kDa.
ab32410 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 10000 dilution for 1 hour at room temperature before imaging.

The expression profile observed in Raji is consistent with the literature (PMID: 25275600).

Negative control: Raji (PMID: 25275600)

All lanes:
Western blot - Anti-ZAP70 antibody [E267] (ab32410) at 1/1000 dilution

Lane 1:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

Lane 2:
Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 69 kDa

---

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.