abcam

ab32429

Anti-ZAP70 antibody [YE291]

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	YE291
Purification technique	Affinity purification Protein A
Concentration	0.162 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

IP

Tested

Species	Human
Dilution info	1/50
Notes	-

WB

Tested

Species	Human
Dilution info	1/500
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/80
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

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	Conditional Ambient
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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.

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

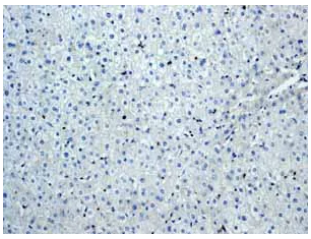
We do not recommend this combination. It is not covered by our product promise.

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.

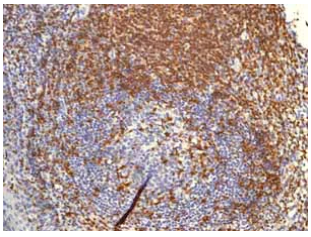
Full details and terms and conditions can be found here:
Terms & Conditions.

14 product images



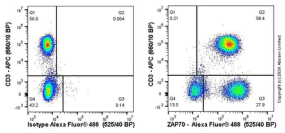
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ZAP70 antibody [YE291] (ab32429)

ab32429 showing negative staining in Normal liver tissue.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ZAP70 antibody [YE291] (ab32429)

ab32429 showing positive staining in Normal tonsil tissue.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



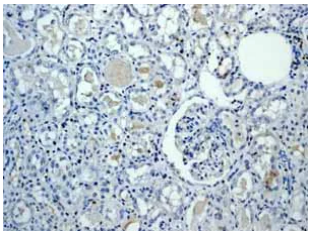
Flow Cytometry (Intracellular) - Anti-ZAP70 antibody [YE291] (ab32429)

ZAP70 Flow Cytometry (Intracellular) staining of Human peripheral blood mononuclear cells using rabbit Anti-ZAP70 antibody

Flow cytometry staining of human peripheral blood mononuclear cells (PBMCs) with ab32429 (right) or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (left). Cells were fixed and permeabilised with BD Cytofix/Cytoperm™ for 20 min. PBMCs were incubated for 30 min on ice in 1x PBS containing 10 µg/ml human IgG and 10 % normal goat serum to block FC receptors and non-specific protein-protein interaction followed by the antibody ab32429 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1x 10^6 in 100 µl at 0.04 µg/ml (1/50000)) for 30 min at 22°C. The cells were simultaneously stained with CD19.

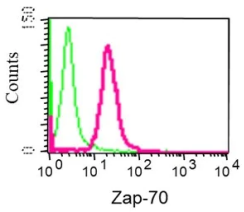
The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C.

Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on live cells.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ZAP70 antibody [YE291] (ab32429)

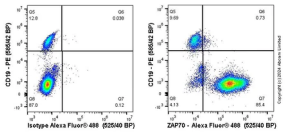
ab32429 showing negative staining in Normal kidney tissue.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-ZAP70 antibody [YE291] (ab32429)

ZAP70 Flow Cytometry (Intracellular) staining using rabbit Anti-ZAP70 antibody

Intracellular Intracellular Intracellular Flow Cytometry analysis of Jurkat cells labeling ZAP70 with ab32429 at 1/80 dilution (red) or rabbit IgG as negative control (green).



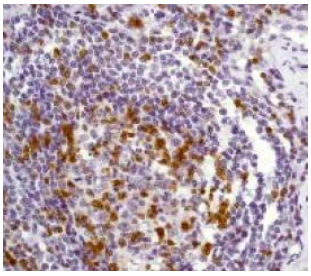
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ZAP70 Flow Cytometry (Intracellular) staining of Human peripheral blood mononuclear cells using rabbit Anti-ZAP70 antibody

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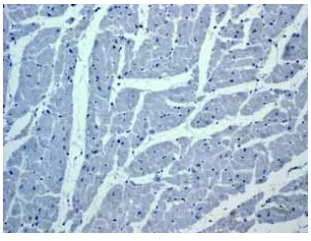
The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Acquisition of >30000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter. Events were gated on live cells.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ZAP70 antibody [YE291] (ab32429)

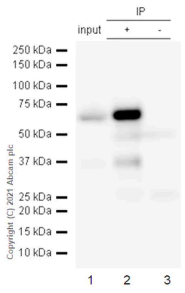
ab32429, at a 1/100 dilution, staining ZAP70 in paraffin embedded human lymph node tissue by immunohistochemistry.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ZAP70 antibody [YE291] (ab32429)

ab32429 showing negative staining in Normal heart tissue.
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Immunoprecipitation - Anti-ZAP70 antibody [YE291] (ab32429)



ZAP70 was immunoprecipitated from 0.35 mg Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10 µg with ab32429 at 1/50 dilution (2µg). VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.
Lane 1: Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10 µg

Lane 2: ab32429 IP in Jurkat whole cell lysate

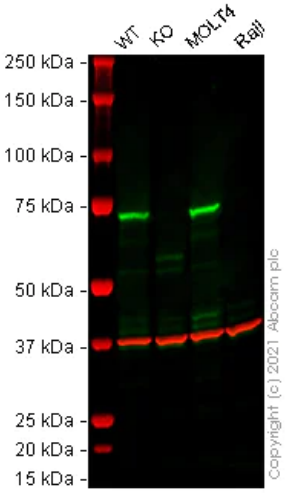
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab32429 in Jurkat whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

All lanes:
Immunoprecipitation - Anti-ZAP70 antibody [YE291] (ab32429)

Predicted band size: 69 kDa

Observed band size: 70 kDa



Western blot - Anti-ZAP70 antibody [YE291] (ab32429)

ZAP70 Western blot staining using rabbit Anti-ZAP70 antibody

False colour image of Western blot: Anti-ZAP70 antibody [YE291] 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, ab32429 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 knockout cell line ab273841 (knockout cell lysate ab273795). The band observed in the knockout 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 knockout 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 [YE291] (ab32429) at 1/500 dilution

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

Lane 2:
ZAP70 knockout Jurkat cell lysate at 20 µg

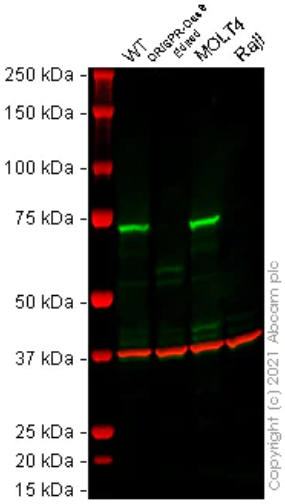
Lane 3:
MOLT-4 cell lysate at 20 µg

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 [YE291] (ab32429)

ZAP70 Western blot staining using rabbit Anti-ZAP70 antibody

False colour image of Western blot: Anti-ZAP70 antibody [YE291] 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, ab32429 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 [YE291] (ab32429) 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 2:
Western blot - Human ZAP70 knockout Jurkat cell line (ab273841)

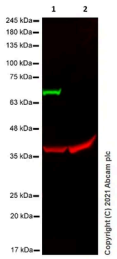
Lane 3:
MOLT-4 cell lysate at 20 µg

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 [YE291] (ab32429)

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 – ab32429 observed at 70 kDa. Red - loading control ab8245 observed at 36 kDa.
ab32429 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 [YE291] (ab32429) at 1/1000 dilution

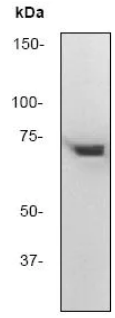
Lane 1:
Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

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



Western blot - Anti-ZAP70 antibody [YE291] (ab32429)

All lanes:
Western blot - Anti-ZAP70 antibody [YE291] (ab32429) at 1/500 dilution

All lanes:
Jurkat cell lysate

Predicted band size: 69 kDa

Observed band size: 70 kDa

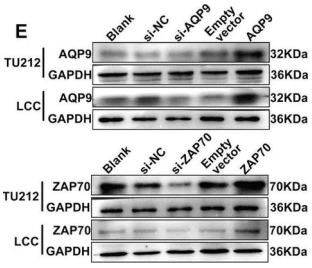


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Western blot - Anti-ZAP70 antibody [YE291] (ab32429)

ZAP70 Western blot staining using rabbit Anti-ZAP70 antibody

ZAP70 western blot using anti-ZAP70 antibody [YE291] ab32429. Publication image and figure legend from Ren, L., Li, P., et al., 2022, BMC Cancer, PubMed 35477402.

ab32429 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab32429 please see the product overview.

AQP9 and ZAP70 up-regulation inhibits cell viability of laryngeal cancer. A, B RT-qPCR for AQP9 and ZAP70 mRNA expression in TU212 and LCC cells transfected with siRNAs against AQP9 and ZAP70 or their overexpression plasmids. C-E Western blot for AQP9 and ZAP70 expression in TU212 and LCC cells transfected with siRNAs against AQP9 and ZAP70 or their overexpression plasmids. F, G CCK-8 for cell viability of TU212 and LCC cells transfected with si-AQP9 or AQP9 overexpression plasmid. H, I CCK-8 for cell viability of TU212 and LCC cells transfected with si-ZAP70 or ZAP70 overexpression plasmid. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

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