

Product datasheet

Anti-ZAP70 antibody [YE291] ab32429

Recombinant RabMAb

[1 References](#) [11 Images](#)

Overview

Product name	Anti-ZAP70 antibody [YE291]
Description	Rabbit monoclonal [YE291] to ZAP70
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P, IP, Flow Cyt (Intra)
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide within Human ZAP70 aa 550 to the C-terminus (C terminal). The exact sequence is proprietary.
Positive control	WB: Jurkat cell lysate. IHC-P: Human lymph node tissue. Flow Cyt (intra): Jurkat cells IP: Jurkat lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	<p>pH: 7.20</p> <p>Preservative: 0.01% Sodium azide</p> <p>Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA</p>
Purity	Protein A purified
Clonality	Monoclonal
Clone number	YE291
Isotype	IgG

Applications

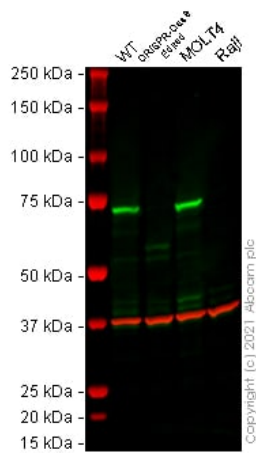
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32429 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/500. Detects a band of approximately 70 kDa (predicted molecular weight: 70 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		1/50.
Flow Cyt (Intra)		1/80. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

Function	Plays a role in T-cell development and lymphocyte activation. Essential for TCR-mediated IL-2 production. Isoform 1 induces TCR-mediated signal transduction, isoform 2 does not.
Tissue specificity	Expressed in T- and natural killer cells.
Involvement in disease	Defects in ZAP70 are the cause of selective T-cell defect (STD) [MIM:176947]. STD is an autosomal recessive form of severe combined immunodeficiency characterized by a selective absence of CD8-type T-cells.
Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. SYK/ZAP-70 subfamily. Contains 1 protein kinase domain. Contains 2 SH2 domains.
Domain	The SH2 domains bind to the phosphorylated tyrosine-based activation motif (TAM) of CD3Z and the non-canonical phosphorylated tyrosine-based activation motif (TAM) of RHOH.
Post-translational modifications	Phosphorylated on tyrosine residues upon T-cell antigen receptor (TCR) stimulation. Tyr-319 phosphorylation is essential for full activity.
Cellular localization	Cytoplasm. Cell membrane. After antigen stimulation, isoform 1 concentrates at the immunological synapse and isoform 2 remains cytoplasmic. Co-localizes together with RHOH in the immunological synapse. RHOH is required for its proper localization to the cell membrane and cytoskeleton fractions in the thymocytes.

Images



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

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

Lane 1 : Wild-type Jurkat cell lysate

Lane 2 : ZAP70 CRISPR-Cas9 edited Jurkat cell lysate

Lane 3 : MOLT-4 cell lysate

Lane 4 : Raji cell lysate

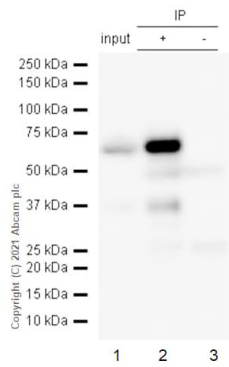
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 70 kDa

Observed band size: 70 kDa

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.



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

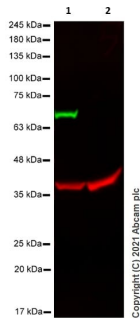
ZAP70 was immunoprecipitated from 0.35 mg Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10 µg with ab32429 at 1/50 dilution (2µg). VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)) was used at 1/5000 dilution.

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.



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

All lanes : Anti-ZAP70 antibody [YE291] (ab32429) at 1/1000 dilution

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

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

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/10000 dilution (Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed)

Predicted band size: 70 kDa

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.

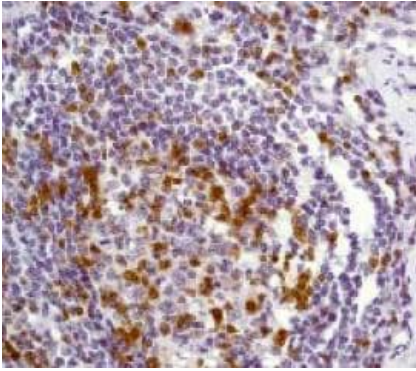
Lanes 1-2: Merged signal (red and green). Green – ab32429 observed at 70 kDa. Red - loading control [ab8245](#) observed at 36 kDa.

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).

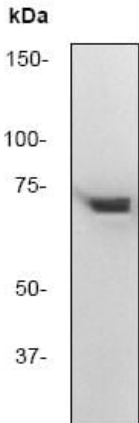
Negative control: Raji (PMID: 25275600)



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.

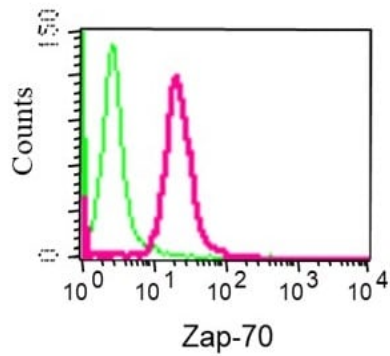


Western blot - Anti-ZAP70 antibody [YE291] (ab32429)

Anti-ZAP70 antibody [YE291] (ab32429) at 1/500 dilution + Jurkat cell lysate

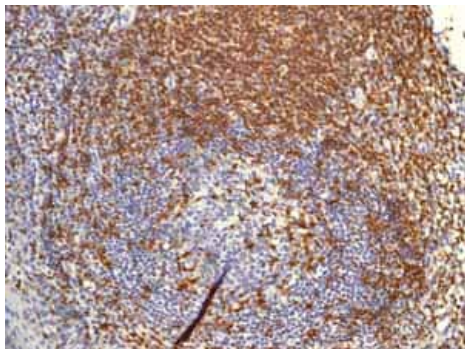
Predicted band size: 70 kDa

Observed band size: 70 kDa



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

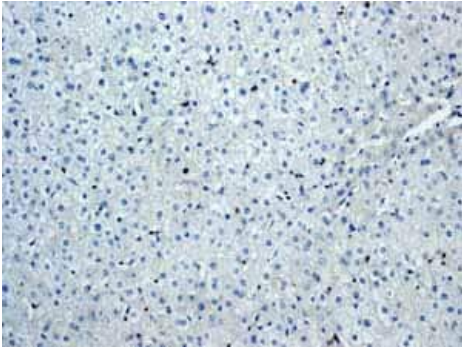
Flow Cytometry (Intracellular) - Anti-ZAP70 antibody [YE291] (ab32429)



ab32429 showing positive staining in Normal tonsil tissue.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

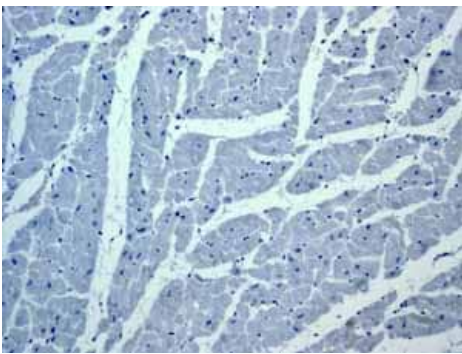
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ZAP70 antibody [YE291] (ab32429)



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

ab32429 showing negative staining in Normal liver tissue.

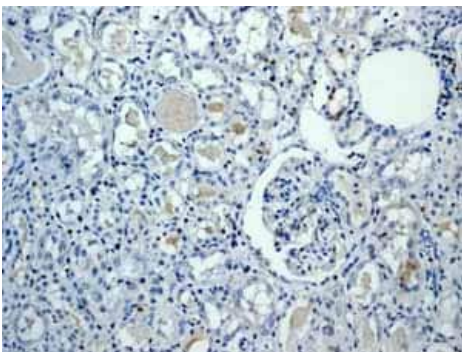
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

ab32429 showing negative staining in Normal heart tissue.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

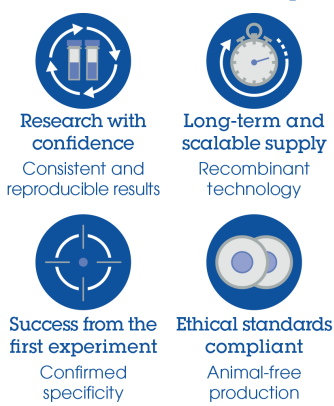


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ZAP70 antibody [YE291] (ab32429)

ab32429 showing negative staining in Normal kidney tissue.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Why choose a recombinant antibody?



- Research with confidence**
Consistent and reproducible results
- Long-term and scalable supply**
Recombinant technology
- Success from the first experiment**
Confirmed specificity
- Ethical standards compliant**
Animal-free production

Anti-ZAP70 antibody [YE291] (ab32429)

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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