abcam

Product datasheet

Anti-ZAP70 antibody [YE291] ab32429

Recombinant RabMAb

4 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	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA
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.

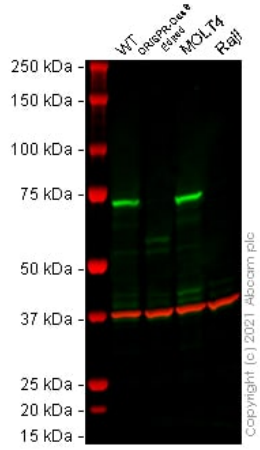
Sequence similarities Belongs to the protein kinase superfamily. Tyr protein kinase family. SYK/ZAP-70 subfamily.
Contains 1 protein kinase domain.
Contains 2 SH2 domains.

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

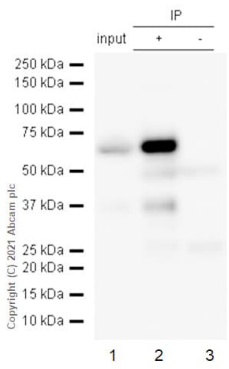
Lane 1 : Wild-type Jurkat cell lysate
Lane 2 : ZAP70 CRISPR-Cas9 edited Jurkat cell lysate
Lane 3 : MOLT-4 cell lysate
Lane 4 : Raji cell lysate

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)

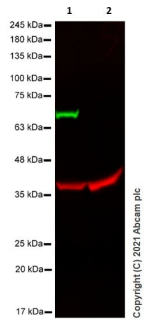
ZAP70 was immunoprecipitated from 0.35 mg Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate 10 µg with ab32429 at 1/50 dilution (2µg). VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

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

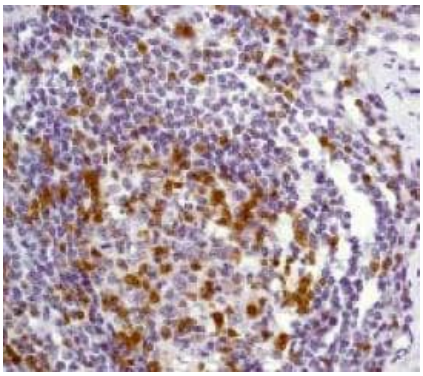
Anti-GAPDH antibody, **ab8245** (1/20000) was used as a primary antibody for the loading control and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed, **ab216776** (1/10000) was used as a loading control secondary antibody.

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).

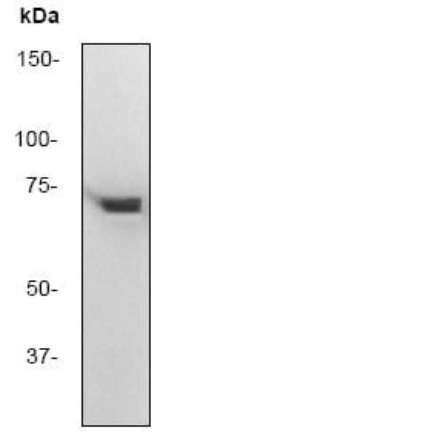
Negative control: Raji (PMID: 25275600)



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.

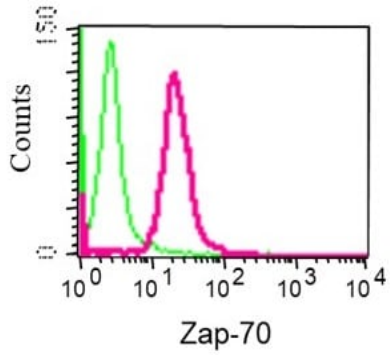
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

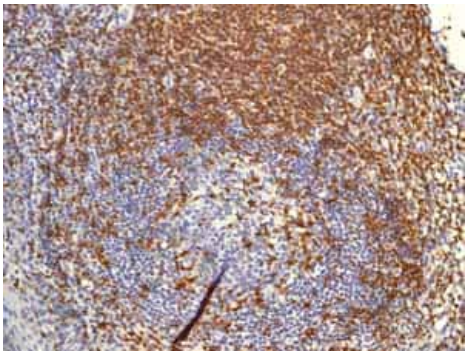
Anti-ZAP70 antibody [YE291] (ab32429) at 1/500 dilution + Jurkat cell lysate

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



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

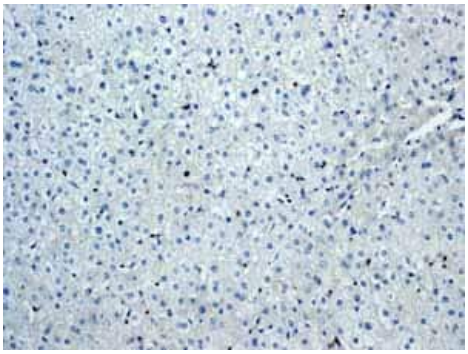
Intracellular Intracellular Intracellular Flow Cytometry analysis of Jurkat cells labeling ZAP70 with ab32429 at 1/80 dilution (red) or rabbit IgG as negative control (green).



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

ab32429 showing positive staining in Normal tonsil tissue.

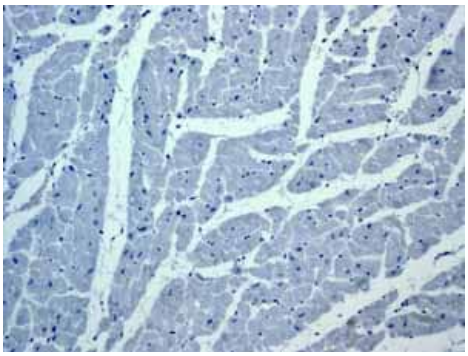
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

ab32429 showing negative staining in Normal liver tissue.

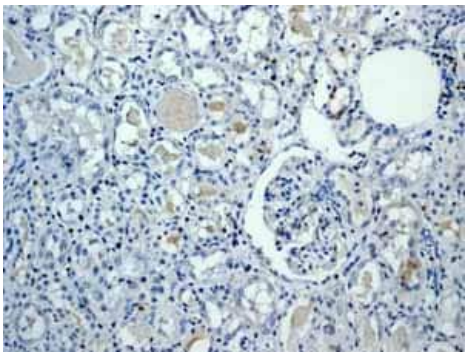
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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

ab32429 showing negative staining in Normal heart tissue.

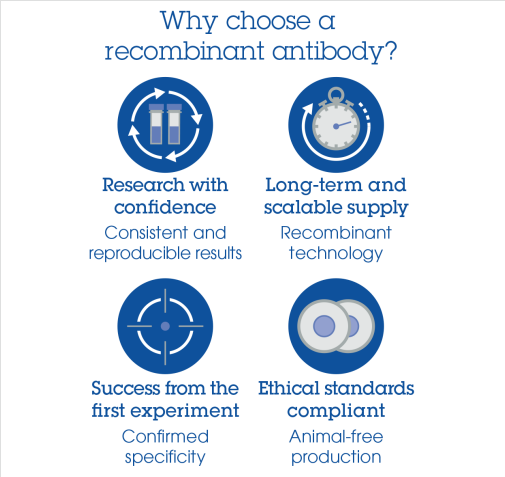
Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.



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.



Anti-ZAP70 antibody [YE291] (ab32429)

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

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit **https://www.abcam.com/abpromise** or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors