abcam

Product datasheet

Anti-ZAP70 antibody [E267] ab32410

Recombinant RabMAb

3 References 4 Images

Overview

Product name Anti-ZAP70 antibody [E267]

Description Rabbit monoclonal [E267] to ZAP70

Host species Rabbit

Specificity ab32410 recognises Zap-70, a Syk-family protein tyrosine kinase.

Tested applications Suitable for: WB

Unsuitable for: ICC/IF or IHC

Reacts with: Human Species reactivity

Predicted to work with: Mouse, Rat

Immunogen Synthetic peptide within Human ZAP70 aa 300-400 (N terminal). The exact sequence is

proprietary.

Positive control Jurkat whole cell lysate (ab7899)

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.

pH: 7.20 Storage buffer

Preservative: 0.01% Sodium azide

Constituents: 49% PBS, 50% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number E267
Isotype IgG

Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab32410 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. Predicted molecular weight: 70 kDa.

Application notes Is unsuitable for ICC/IF or IHC.

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 similaritiesBelongs 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

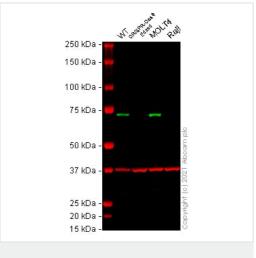
phosphorylation is essential for full activity.

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 [E267] (ab32410)

All lanes: Anti-ZAP70 antibody [E267] (ab32410) 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 [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 lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat

anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.

1 2
245 kDa=
190 kDa=
135 kDa=
100 kDa=
75 kDa=
63 kDa=
48 kDa=
35 kDa=
25 kDa=
20 kDa

(ab32410)

All lanes : Anti-ZAP70 antibody [E267] (ab32410) 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 lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution (Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed)

Predicted band size: 70 kDa

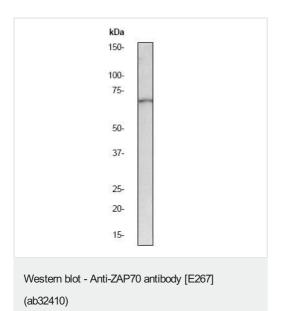
Anti-GAPDH antibody, <u>ab8245</u> (1/20000) was used as a primary antibody for the loading control and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed, <u>ab216776</u> (1/10000) was used as a loading control secondary antibody.

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 lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG 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)



Anti-ZAP70 antibody [E267] (ab32410) at 1/500 dilution + Jurkat cell lysates

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

Western blot analysis on Jurkat Cell lysate.



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