abcam

Product datasheet

Anti-ZAP70 antibody [E267] - BSA and Azide free ab247256

Recombinant RabMAb

4 Images

Overview

Product name	Anti-ZAP70 antibody [E267] - BSA and Azide free
Description	Rabbit monoclonal [E267] to ZAP70 - BSA and Azide free
Host species	Rabbit
Specificity	This antibody recognises Zap-70, a Syk-family protein tyrosine kinase.
Tested applications	**Suitable for:** WB **Unsuitable for:** ICC/IF or IHC
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
General notes	ab247256 is the carrier-free version of **ab32410**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E267
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab247256 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		Use at an assay dependent concentration. Predicted molecular weight: 70 kDa.

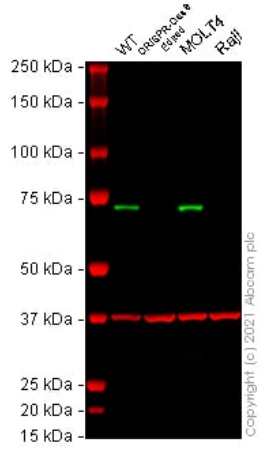
Application notes Is unsuitable for ICC/IF or IHC.

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 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 [E267] - BSA and Azide free (ab247256)

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

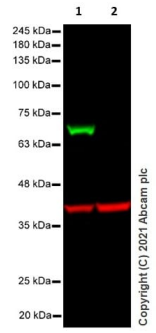
Lane 1 : Wild-type Jurkat cell lysate
Lane 2 : ZAP70 CRISPR-Cas9 edited Jurkat cell lysate
Lane 3 : MOLT-4 cell lysate
Lane 4 : Raji cell lysate

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 IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



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

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

All lanes :

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-Rabbit IgG H&L (IRDye® 800CW) preadsorbed)

Predicted band size: 70 kDa

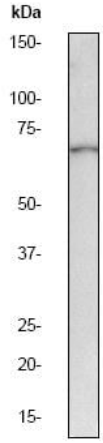
Anti-GAPDH antibody, **ab8245** (1/20000) was used as a primary antibody for the loading control and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed, **ab216776** (1/10000) was used as a loading control secondary antibody.

Lanes 1-2: Merged signal (red and green). Green – **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)



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

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

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

This data was developed using **ab32410**, the same antibody clone in a different buffer formulation.

Western blot analysis on Jurkat Cell lysate.

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

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