abcam

ab325911

Anti-ZAP70 antibody [EPR29942-36]

Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR29942-36
Purification technique	Affinity purification Protein A
Specificity	Evidence for nuclear ZAP70: https://www.nature.com/articles/3800145.pdf https://pmc.ncbi.nlm.nih.gov/articles/PMC2199132/

Reactivity data

Flow Cyt

Tested

Species	Mouse
Dilution info	1 µg/mL
Notes	-

Species	Human
Dilution info	0.2 µg/mL
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	1 µg/mL
Notes	-

Species	Human
Dilution info	1 µg/mL
Notes	-

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:10952314). 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-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 duration	1-2 weeks
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.

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

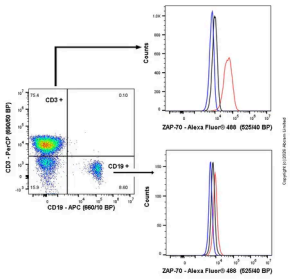
We do not recommend this combination. It is not covered by our product promise.

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.

6 product images



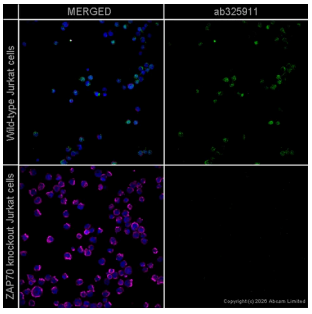
Flow Cytometry - Anti-ZAP70 antibody [EPR29942-36] (ab325911)

ZAP70 Flow Cytometry staining using rabbit Anti-ZAP70 antibody

Flow cytometry overlay histogram showing top CD3⁺ human PBMCs (positive cells) and bottom CD19⁺ human PBMCs (negative cells) stained with ab325911 (red line). Cells were fixed and permeabilised with BD Cytofix/Cytoperm for 20 min. Cells were incubated for 30 min on ice in 1× PBS containing 10 µg/ml human IgG and 10% normal goat serum to block Fc receptors and non-specific protein-protein interactions, followed by the antibody ab325911 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1×10^6 cells in 100 µl at 0.2 µg/ml) for 30 min at 22°C. The cells were simultaneously stained with CD3 and CD19.

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control, used at the same concentration and under the same conditions as the primary antibody. An unlabelled sample (without incubation with the primary antibody, blue line) was also used as a control. The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor 488), preadsorbed, was incubated at 1/4000 for 30 min at 22°C.

Acquisition of >30, 000 events was performed using a 50 mW blue laser (488 nm) and a 525/40 bandpass filter. Events were gated on either CD3⁺ or CD19⁺ cells.

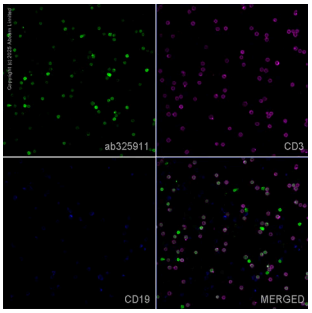


Immunocytochemistry/ Immunofluorescence - Anti-ZAP70 antibody [EPR29942-36] (ab325911)

ZAP70 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ZAP70 antibody

ab325911 staining ZAP70 in Jurkat WT/KO cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with Tween-20 for 5 minutes and then blocked with 1% BSA/10% normal rabbit serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab325911 at 1 µg/mL (shown in green) and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control (shown in pseudocolour magenta). Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor 488), pre-adsorbed at 1/1000 dilution. Nuclear DNA was labelled with DAPI (shown in blue). Also suitable in cells fixed with 100% methanol (5 min). Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a single confocal section is shown.

A subpopulation of ZAP70 positive cells display nuclear subcellular staining, as shown in PMID: 9362531 and PMID: 15133473.

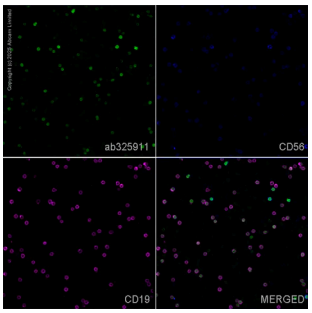


Immunocytochemistry/ Immunofluorescence - Anti-ZAP70 antibody [EPR29942-36] (ab325911)

ZAP70 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ZAP70 antibody

ab325911 staining ZAP70 in Human PBMCs cells. The cells were fixed with BD Cytofix/Cytoperm™ for 20 min, permeabilized with for 5 minutes and then incubated in 1× PBS containing 10 µg/ml human IgG and 10% normal goat serum to block Fc receptors and non-specific protein-protein interactions in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab325911 at 1µg/mL (shown in green), anti-human CD3 (APC) (shown in pseudocolour magenta) and anti-human CD19 (PE) (shown in pseudocolour blue). Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor 488), pre-adsorbed at 1/1000 dilution. Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a single confocal section is shown.

ZAP70 is expressed on CD3 positive PBMCs, whilst ZAP70 and CD19 expression is mutually exclusive. A subpopulation of ZAP70 positive PBMCs display nuclear subcellular staining, as shown in PMID: 9362531 and PMID: 15133473.

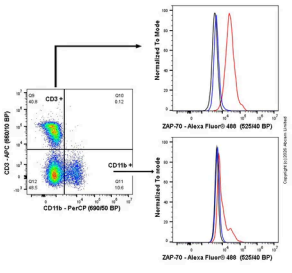


Immunocytochemistry/ Immunofluorescence - Anti-ZAP70 antibody [EPR29942-36] (ab325911)

ZAP70 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ZAP70 antibody

ab325911 staining ZAP70 in Human PBMCs cells. The cells were fixed with BD Cytofix/Cytoperm™ for 20 min, permeabilized with for 5 minutes and then incubated in 1× PBS containing 10 µg/ml human IgG and 10% normal goat serum to block Fc receptors and non-specific protein-protein interactions for 1h. The cells were then incubated overnight at 4°C with ab325911 at 1 µg/mL (shown in green), anti-human CD19 (APC) (shown in pseudocolour magenta) and anti-human CD56 (PE) (shown in pseudocolour blue). Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor 488), pre-adsorbed at 1/1000 dilution. Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a single confocal section is shown.

ZAP70 is expressed on CD56 positive PBMCs, whilst ZAP70 and CD19 expression is mutually exclusive. A subpopulation of ZAP70 positive PBMCs display nuclear subcellular staining, as shown in PMID: 9362531 and PMID: 15133473.



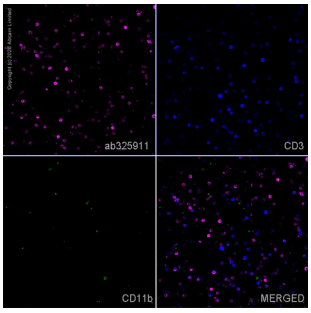
Flow Cytometry - Anti-ZAP70 antibody [EPR29942-36] (ab325911)

ZAP70 Flow Cytometry staining using rabbit Anti-ZAP70 antibody

Flow cytometry overlay histogram showing top CD3⁺ C57BL/6 mouse splenocytes (positive cells) and bottom CD11b⁺ C57BL/6 mouse splenocytes (negative cells) stained with ab325911 (red line). Cells were fixed and permeabilised with BD Cytofix/Cytoperm for 20 min. Cells were incubated for 30 min on ice in 1× PBS containing 10 µg/ml anti-CD16/CD32 and 10% normal goat serum to block Fc receptors and non-specific protein-protein interactions, followed by the antibody ab325911 or Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (1×10^6 cells in 100 µl at 1µg/ml) for 30 min at 22°C. The cells were simultaneously stained with CD3 and CD11b.

Isotype control antibody (black line) was Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control, used at the same concentration and under the same conditions as the primary antibody. An unlabelled sample (without incubation with the primary antibody, blue line) was also used as a control. The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor 488), preadsorbed, was incubated at 1/4000 for 30 min at 22°C.

Acquisition of >30, 000 events was performed using a 50 mW blue laser (488 nm) and a 525/40 bandpass filter. Events were gated on either $CD3^+$ or $CD11b^+$ cells.



Immunocytochemistry/ Immunofluorescence - Anti-ZAP70 antibody [EPR29942-36] (ab325911)

ZAP70 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-ZAP70 antibody

ab325911 staining ZAP70 in mouse splenocytes cells. The cells were fixed and permeabilized with BD Cytofix/Cytoperm™ for 20 min and then incubated in 1× PBS containing 10 µg/ml anti-CD16/CD32 and 10% normal goat serum to block Fc receptors and non-specific protein-protein interactions for 1 hr. The cells were then incubated overnight at 4°C with ab325911 at 1 µg/mL (shown in pseudocolour magenta), anti-mouse CD3 (PE) at 1/2000 dilution (shown in pseudocolour blue) and anti-mouse/human CD11b (Alexa Fluor 488) (shown in green) at 1/500 dilution. Cells were then incubated with ab150083 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 647) at 1/1000 dilution.
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a single confocal section is shown.

ZAP70 is expressed on CD3 positive splenocytes, whilst ZAP70 and CD11b expression is mutually exclusive. A subpopulation of ZAP70 positive splenocytes display nuclear subcellular staining, as shown in PMID: 9362531 and PMID: 15133473.

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.