abcam

ab300398

Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39]

Recombinant | RabMAb | 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	EPR26232-39
Purification technique	Affinity purification Protein A
Concentration	0.472 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse
Dilution info	1/1000
Notes	-

Not recommended

Species	Rat, Synthetic peptide - Human, Synthetic peptide - Mouse
Dilution info	-
Notes	-

Dot

Tested

Species	Synthetic peptide - Human
Dilution info	1/1000
Notes	-

Species	Synthetic peptide - Mouse
Dilution info	1/1000
Notes	-

Expected

Species	Human, Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/500
Notes	Not suitable for Human Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Human
Dilution info	-
Notes	Not suitable for Human Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	-
Notes	Not suitable for Human Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Synthetic peptide - Human
Dilution info	-
Notes	-

Species	Synthetic peptide - Mouse
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

Species	Mouse
Dilution info	1/50
Notes	-

Not recommended

Species	Rat, Synthetic peptide - Human, Synthetic peptide - Mouse
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50
Notes	-

Species	Mouse
Dilution info	1/50
Notes	-

Not recommended

Species	Rat, Synthetic peptide - Human, Synthetic peptide - Mouse
Dilution info	-
Notes	-

IP

Not recommended

Species	Human, Mouse, Rat, Synthetic peptide - Human, Synthetic peptide - Mouse
Dilution info	-
Notes	-

Target data

See full target information ZAP70 pY319

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:7509083). 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-CD247/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	Do Not Freeze

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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	Syk (spleen tyrosine kinase) and ZAP70 (zeta-chain-associated protein kinase 70) are non-receptor tyrosine kinases. Syk has a molecular mass of approximately 72 kDa while ZAP70 is around 70 kDa. They are expressed in hematopoietic tissues. Syk is found in B-cells and other myeloid lineages and ZAP70 is mainly located in T-cells and natural killer (NK) cells. These kinases play essential roles in signal transduction by phosphorylating proteins on tyrosine residues transmitting activation signals from receptors on the cell surface to the interior of the cell.
Biological function summary	Syk and ZAP70 play vital roles in the adaptive immune response. Syk is an essential mediator of B-cell receptor (BCR) signaling and assembles into a signaling complex with other proteins. ZAP70 functions similarly in T-cells becoming activated upon the engagement of the T-cell receptor (TCR). Both kinases help recruit and activate adaptor proteins and downstream signaling molecules that drive cellular responses including proliferation differentiation and survival.
Pathways	Both kinases are integral to the B-cell and T-cell receptor signaling pathways. Syk activates several pathways including the MAP kinase and NF-kB pathways through its interaction with proteins like BLNK and PLCγ2. ZAP70 initiates the TCR signaling cascade interfacing with LAT and SLP-76 to activate the same pathways. These pathways trigger transcriptional programs necessary for immune cell functions and development.
Associated diseases and disorders	Syk and ZAP70 have connections to various immune-related conditions. Abnormal Syk activity relates to autoimmune diseases like rheumatoid arthritis where B-cell signaling plays a role. Syk interacts with proteins such as ITAM-containing immunoreceptors in these disorders. ZAP70 mutations are linked to immunodeficiency syndromes leading to dysfunctional T-cell signaling. These kinases make viable targets for therapeutic intervention bringing hope for better management of such immune diseases.

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.

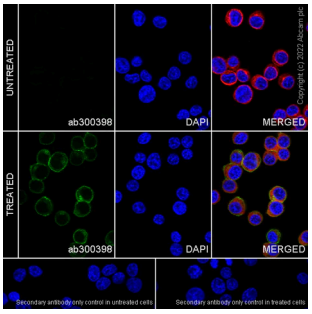
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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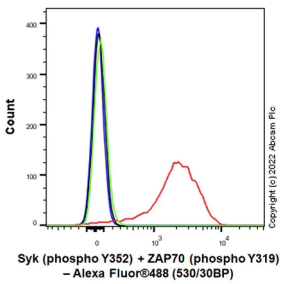
9 product images



Immunocytochemistry/ Immunofluorescence - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398)

Syk (phospho Y352) +ZAP70 (phospho Y319) Immunocytochemistry/ Immunofluorescence staining of Jurkat cells treated with pervanadate (50 mM) for 5 min using rabbit Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized Jurkat (human T cell leukemia T lymphocyte) cells labelling Syk (phospho Y352) +ZAP70 (phospho Y319) with ab300398 at 1/50 (9.44 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/ml dilution (Green). Confocal image showing increased membranous staining in Jurkat cells treated with pervanadate (50 mM) for 5 min is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/ml dilution.

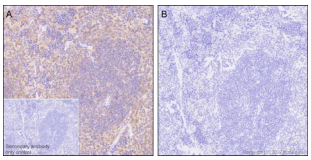


Flow Cytometry (Intracellular) - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398)

Syk (phospho Y352) +ZAP70 (phospho Y319) Flow Cytometry (Intracellular) staining of 4% paraformaldehyde fixed 90% methanol permeabilized Jurkat (human T cell leukemia cell line from peripheral blood) treated with 1mM pervanadate for 30 minutes using rabbit Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized Jurkat (human T cell leukemia cell line from peripheral blood) treated with 1mM pervanadate for 30 minutes (Red)/ Untreated control (Green) cells labelling Syk (phospho Y352) +ZAP70 (phospho

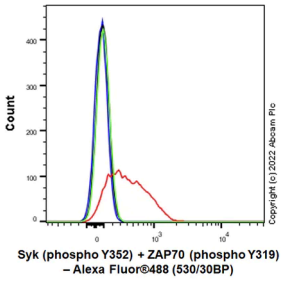
Y319) with ab300398 at 1/500 dilution (0.1ug) (Red) and Green (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398)

Syk (phospho Y352) +ZAP70 (phospho Y319) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse spleen using rabbit Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody

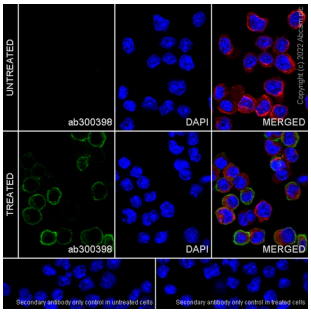
Immunohistochemical analysis of paraffin-embedded Panel A, Mouse splee tissue labeling Syk (phospho Y352) +ZAP70 (phospho Y319) with ab300398 at 1/500 (0.944 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse spleen without alkaline phosphatase treatment (Panel A); no staining on mouse spleen with alkaline phosphatase treatment (Panel B). The section was incubated with ab300398 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Flow Cytometry (Intracellular) - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398)

Syk (phospho Y352) +ZAP70 (phospho Y319) Flow Cytometry (Intracellular) staining of 4% paraformaldehyde fixed 90% methanol permeabilized EL4 (mouse lymphoma t lymphocyte) treated with 11mM H2O2 for 10 minutes then 10mM pervanadate for 30 minutes using rabbit Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized EL4 (mouse lymphoma t lymphocyte) treated with 11mM H2O2 for 10 minutes then 10mM pervanadate for 30 minutes (Red)/ Untreated control (Green) cells labelling Syk (phospho Y352) +ZAP70 (phospho Y319) with ab300398 at 1/50 dilution (1ug) (Red) and Green (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.

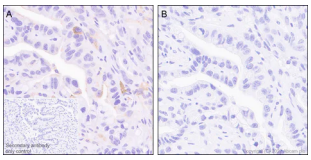


Immunocytochemistry/ Immunofluorescence - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398)

Syk (phospho Y352) +ZAP70 (phospho Y319) Immunocytochemistry/ Immunofluorescence staining of EL4 (mouse lymphoma T lymphocyte) using rabbit Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized EL4 (mouse lymphoma T lymphocyte) cells labelling Syk (phospho Y352) +ZAP70 (phospho Y319) with ab300398 at 1/50 (9.44 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2 ug/ml dilution (Green). Confocal image showing increased membranous staining in EL4 cells treated with H2O2 (11 mM) for 10 min and then pervanadate (10 mM) for 30 min is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5ug/ml dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only

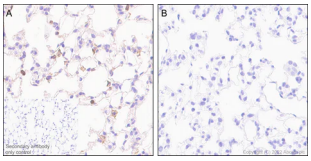
control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/ml dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398)

Syk (phospho Y352) +ZAP70 (phospho Y319) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse pancreatic cancer using rabbit Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody

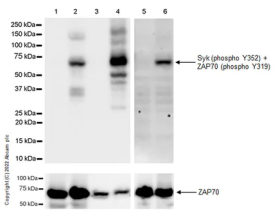
Immunohistochemical analysis of paraffin-embedded Panel A, Mouse pancr tissue labeling Syk (phospho Y352) +ZAP70 (phospho Y319) with ab300398 at 1/500 (0.944 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse pancreatic cancer without alkaline phosphatase treatment (Panel A); no staining on mouse pancreatic cancer with alkaline phosphatase treatment (Panel B). The section was incubated with ab300398 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398)

Syk (phospho Y352) +ZAP70 (phospho Y319) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse lung using rabbit Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody

Immunohistochemical analysis of paraffin-embedded Panel A, Mouse lung tissue labeling Syk (phospho Y352) +ZAP70 (phospho Y319) with ab300398 at 1/500 (0.944 ug/ml) followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse lung without alkaline phosphatase treatment (Panel A); no staining on mouse lung with alkaline phosphatase treatment (Panel B). The section was incubated with ab300398 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Western blot - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398)

Syk (phospho Y352) +ZAP70 (phospho Y319) Western blot staining using rabbit Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody

Blocking / Diluting buffer and concentration:5% NFDM/TBST

Exposure time: Lanes 1-4: 6 seconds; Lanes 5-6: 147 seconds.

All lanes:
Western blot - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398) at 1/1000 dilution

Lane 1:
Untreated Jurkat whole cell lysate at 20 µg

Lane 2:
Jurkat treated with 50mM pervanadate for 5 minutes whole cell lysate at 20 µg

Lane 3:
ntreated starved 24 hours Ramos whole cell lysate at 20 µg

Lane 4:
Ramos starved 24 hours, then treated with 10mM pervanadate for 30 minuteswhole cell lysate at 20 µg

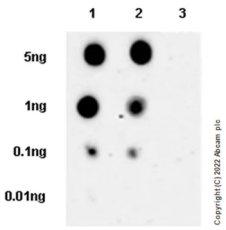
Lane 5:
Untreated EL4 whole cell lysate at 20 µg

Lane 6:
EL4 treated with 11mM H2O2 for 10 minutes, then 10mM pervanadate for 30 minutes whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Observed band size: 70 kDa, 72 kDa



Dot Blot - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398)

Syk (phospho Y352) +ZAP70 (phospho Y319) Dot Blot staining using rabbit Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody

Blocking/diluting buffer and concentration: 5% NFDM/TBST

All lanes:
Dot Blot - Anti-Syk (phospho Y352) +ZAP70 (phospho Y319) antibody [EPR26232-39] (ab300398) at 1/1000 dilution

Lane 1:
Syk (phospho Y352) peptide a

Lane 2:
Syk (phospho Y352) peptide b

Lane 3:
Syk non-phospho peptide

Secondary

All lanes:
Dot Blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

Exposure time: 180s

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