

Product datasheet

Anti-Syk (phospho Y323) antibody [EP573-4] - Low endotoxin, Azide free ab217708

Recombinant RabMAb

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Overview

Product name	Anti-Syk (phospho Y323) antibody [EP573-4] - Low endotoxin, Azide free
Description	Rabbit monoclonal [EP573-4] to Syk (phospho Y323) - Low endotoxin, Azide free
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P, Flow Cyt (Intra), ICC/IF, Dot blot Unsuitable for: IP
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: U937 cell lysates treated with pervanadate. IHC-P: Human lymph node tissue.
General notes	<p>ab217708 is the carrier-free version of ab62338.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>Our Low endotoxin, azide-free formats have low endotoxin level (≤ 1 EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.</p> <p>Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.</p>

Properties

Form Liquid

Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP573-4
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab217708 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. Detects a band of approximately 72 kDa (predicted molecular weight: 72 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.
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG (Low endotoxin, Azide free), is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
Dot blot		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

Target

Function Non-receptor tyrosine kinase which mediates signal transduction downstream of a variety of transmembrane receptors including classical immunoreceptors like the B-cell receptor (BCR). Regulates several biological processes including innate and adaptive immunity, cell adhesion, osteoclast maturation, platelet activation and vascular development. Assembles into signaling complexes with activated receptors at the plasma membrane via interaction between its SH2 domains and the receptor tyrosine-phosphorylated ITAM domains. The association with the receptor can also be indirect and mediated by adapter proteins containing ITAM or partial hemITAM domains. The phosphorylation of the ITAM domains is generally mediated by SRC subfamily kinases upon engagement of the receptor. More rarely signal transduction via SYK could be ITAM-independent. Direct downstream effectors phosphorylated by SYK include VAV1, PLCG1, PI-3-kinase, LCP2 and BLNK. Initially identified as essential in B-cell receptor (BCR) signaling, it is necessary for the maturation of B-cells most probably at the pro-B to pre-B transition. Activated upon BCR engagement, it phosphorylates and activates BLNK an adapter linking the activated BCR to downstream signaling adapters and effectors. It also phosphorylates

and activates PLCG1 and the PKC signaling pathway. It also phosphorylates BTK and regulates its activity in B-cell antigen receptor (BCR)-coupled signaling. In addition to its function downstream of BCR plays also a role in T-cell receptor signaling. Plays also a crucial role in the innate immune response to fungal, bacterial and viral pathogens. It is for instance activated by the membrane lectin CLEC7A. Upon stimulation by fungal proteins, CLEC7A together with SYK activates immune cells inducing the production of ROS. Also activates the inflammasome and NF-kappa-B-mediated transcription of chemokines and cytokines in presence of pathogens. Regulates neutrophil degranulation and phagocytosis through activation of the MAPK signaling cascade. Also mediates the activation of dendritic cells by cell necrosis stimuli. Also involved in mast cells activation. Also functions downstream of receptors mediating cell adhesion. Relays for instance, integrin-mediated neutrophils and macrophages activation and P-selectin receptor/SELPG-mediated recruitment of leukocytes to inflammatory loci. Plays also a role in non-immune processes. It is for instance involved in vascular development where it may regulate blood and lymphatic vascular separation. It is also required for osteoclast development and function. Functions in the activation of platelets by collagen, mediating PLCG2 phosphorylation and activation. May be coupled to the collagen receptor by the ITAM domain-containing FCER1G. Also activated by the membrane lectin CLEC1B that is required for activation of platelets by PDPN/podoplanin. Involved in platelet adhesion being activated by ITGB3 engaged by fibrinogen.

Tissue specificity

Widely expressed in hematopoietic cells (at protein level). Within the B-cells compartment it is for instance expressed for pro-B-cells to plasma 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 mediate the interaction of SYK with the phosphorylated ITAM domains of transmembrane proteins. Some proteins like CLEC1B have a partial ITAM domain (also called hemITAM) containing a single YxxL motif. The interaction with SYK requires CLEC1B homodimerization.

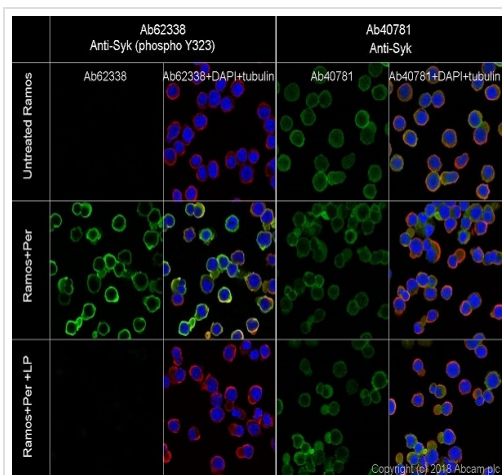
Post-translational modifications

Ubiquitinated by CBLB after BCR activation; which promotes proteasomal degradation. Autophosphorylated. Phosphorylated on tyrosine residues by LYN following receptors engagement. Phosphorylation on Tyr-323 creates a binding site for CBL, an adapter protein that serves as a negative regulator of BCR-stimulated calcium ion signaling. Phosphorylation at Tyr-348 creates a binding site for VAV1. Phosphorylation on Tyr-348 and Tyr-352 enhances the phosphorylation and activation of phospholipase C-gamma and the early phase of calcium ion mobilization via a phosphoinositide 3-kinase-independent pathway (By similarity). Phosphorylation on Ser-297 is very common, it peaks 5 minutes after BCR stimulation, and creates a binding site for YWHAG. Phosphorylation at Tyr-630 creates a binding site for BLNK. Dephosphorylated by PTPN6.

Cellular localization

Cell membrane. Cytoplasm, cytosol.

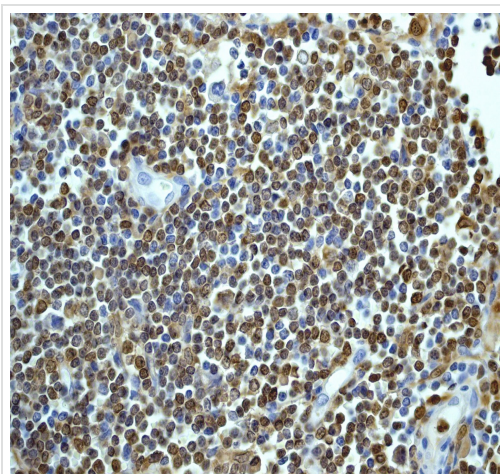
Images



Immunocytochemistry/ Immunofluorescence - Anti-Syk (phospho Y323) antibody [EP573-4] - Low endotoxin, Azide free (ab217708)

Immunocytochemistry/Immunofluorescence analysis of Ramos+/- pervanadate(1mM, 30min), Ramos + pervanadate(1mM, 30min) + LP labelling Syk (phospho Y323) with **ab62338** at a dilution of 1:1000 dilution (2.53ug/ml). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. **ab150077** Goat anti rabbit IgG(Alexa Fluor® 488) (1:1000) was used as the secondary antibody. The cells were co-stained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). Nuclei counterstained with DAPI (blue). Control: PBS instead of the primary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab62338**).

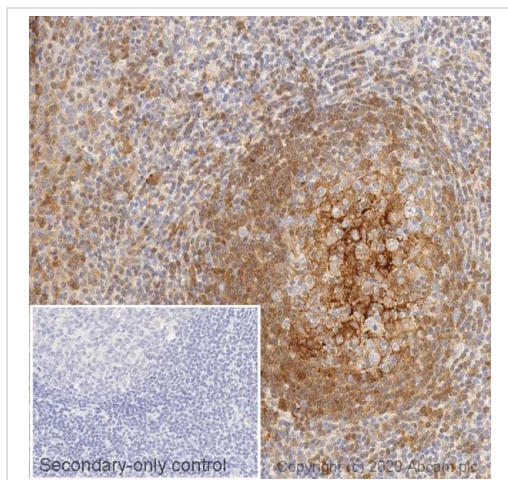


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Syk (phospho Y323) antibody [EP573-4] - Low endotoxin, Azide free (ab217708)

This data was developed using the same antibody clone in a different buffer formulation (**ab62338**).

Immunohistochemical analysis of paraffin-embedded human lymph node tissue sections labeling Syk with purified **ab62338** at 1/100 dilution. ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Sections were counterstained with Hematoxylin.

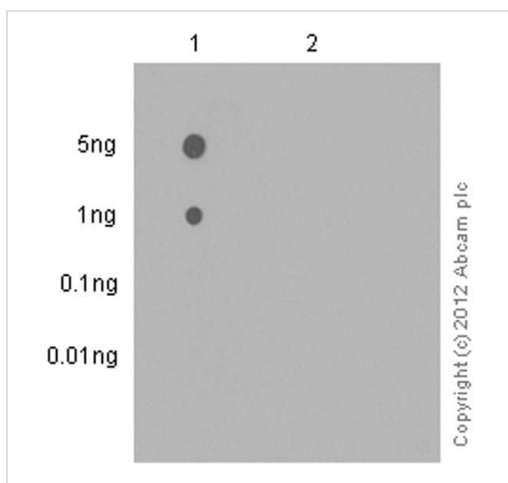
Antigen retrieval was heat mediated antigen retrieval using citrate buffer, pH 6.0).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Syk (phospho Y323) antibody [EP573-4] - Low endotoxin, Azide free (ab217708)

IHC image of Syk (phospho Y323) staining in a section of formalin-fixed paraffin-embedded normal human tonsil* performed on a Leica BOND™ system using the standard protocol **F**. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with **ab70328**, 0.05ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



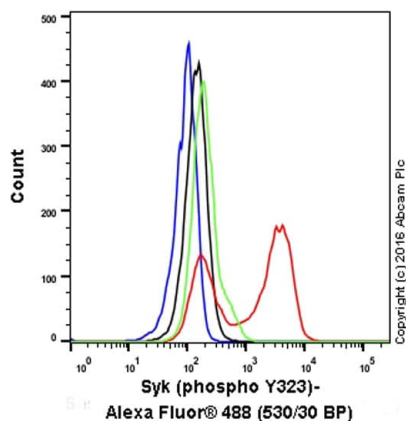
Dot Blot - Anti-Syk (phospho Y323) antibody [EP573-4] - Low endotoxin, Azide free (ab217708)

Dot blot analysis of Sky (pY323) peptide (Lane 1), Syk (unmodified) peptide (Lane 2), labelling Syk (pY323) with **ab62338** at a dilution of 1/1000. Peroxidase conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody at a dilution of 1/2500.

Blocking and dilution buffer: 5% NFDm/TBST.

Exposure time: 10 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab62338**).

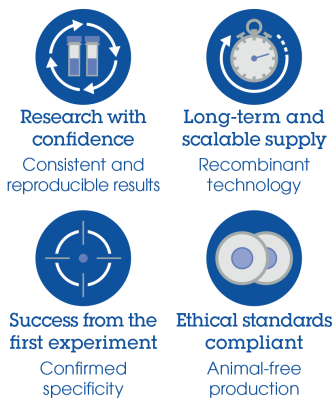


Flow Cytometry (Intracellular) - Anti-Syk (phospho Y323) antibody [EP573-4] - Low endotoxin, Azide free (ab217708)

This Flow cyt data was generated using the same anti-phospho Syk Y323 antibody clone, EP573-4, in a different buffer formulation (cat **ab62338**).

Intracellular Flow Cytometry analysis of U937 (human monocyte histiocytic lymphoma) treated (red)/untreated (green) with 1mM pervanadate for 30 minutes with purified **ab62338** at 1/2500 dilution. The secondary antibody was Goat anti rabbit IgG (Alexa Fluor®488) at 1/2000 dilution. A Rabbit monoclonal IgG (Black) was used as the isotype control and cells without incubation with primary antibody and secondary antibody (Blue) were used as unlabeled control.

Why choose a recombinant antibody?



Anti-Syk (phospho Y323) antibody [EP573-4] - Low endotoxin, Azide free (ab217708)

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