abcam

Product datasheet

Anti-Syk (phospho Y323) antibody [EP573-4] ab62338

Recombinant RabMAb

8 References 7 Images

Overview

Product name	Anti-Syk (phospho Y323) antibody [EP573-4]
Description	Rabbit monoclonal [EP573-4] to Syk (phospho Y323)
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), Dot blot, WB, IHC-P, ICC/IF **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	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**. Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP573-4
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab62338 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
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
Dot blot		1/1000.
WB		1/1000 - 1/2000. Detects a band of approximately 72 kDa (predicted molecular weight: 72 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
ICC/IF		1/100 - 1/250.

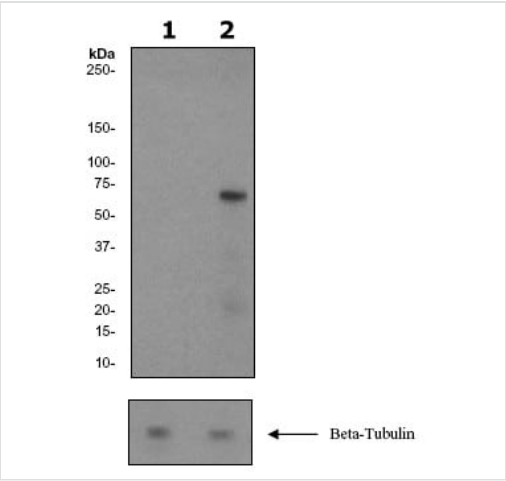
Application notes Is unsuitable for IP.

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



Western blot - Anti-Syk (phospho Y323) antibody [EP573-4] (ab62338)

All lanes : Anti-Syk (phospho Y323) antibody [EP573-4] (ab62338) at 1/2000 dilution

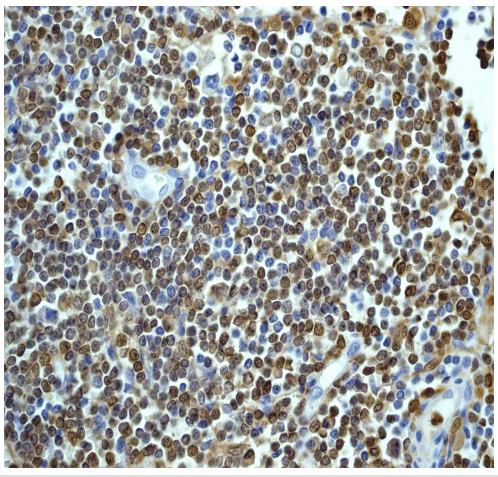
Lane 1 : untreated U937 cell lysates
Lane 2 : U937 cell lysates treated with pervanadate.

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : goat anti-rabbit, HRP labeled, at 1/2000 dilution

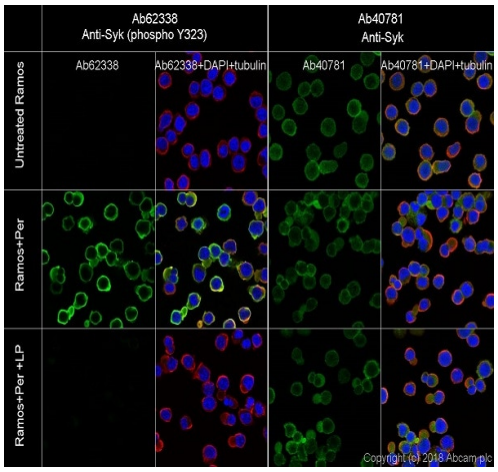
Predicted band size: 72 kDa
Observed band size: 72 kDa

Beta tubulin shows equal lysate loading amount per lane.



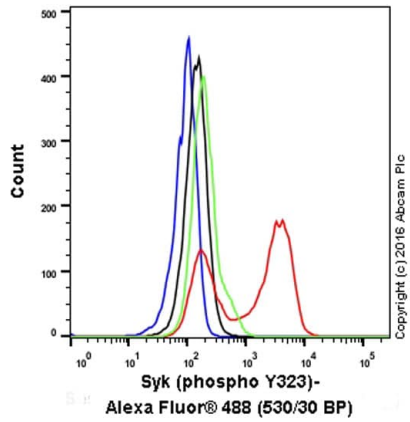
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Syk (phospho Y323) antibody [EP573-4] (ab62338)

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).



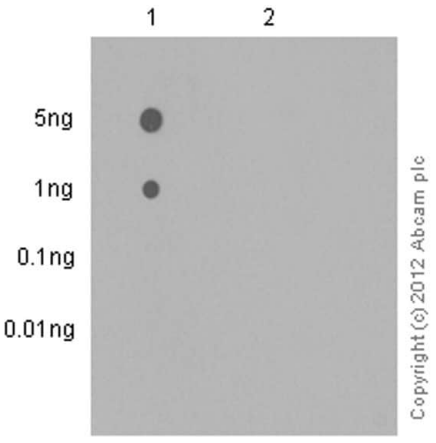
Immunocytochemistry/ Immunofluorescence - Anti-Syk (phospho Y323) antibody [EP573-4] (ab62338)

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.



Flow Cytometry (Intracellular) - Anti-Syk (phospho Y323) antibody [EP573-4] (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 Fluorr® 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.

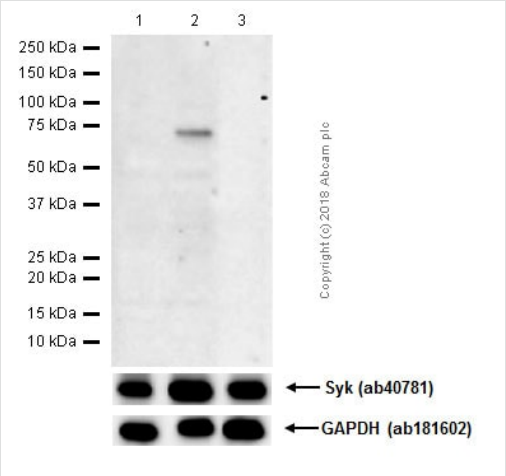


Dot Blot - Anti-Syk (phospho Y323) antibody [EP573-4] (ab62338)

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.



Western blot - Anti-Syk (phospho Y323) antibody [EP573-4] (ab62338)

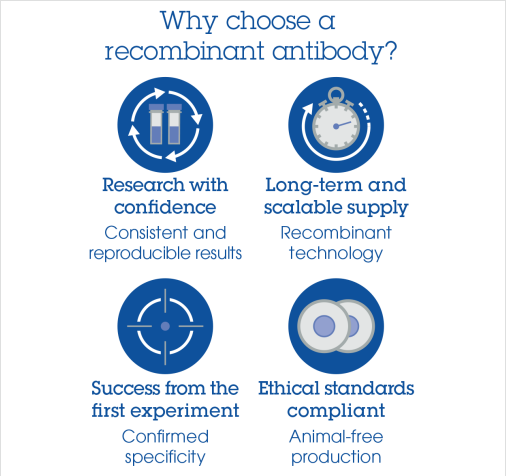
All lanes : Anti-Syk (phospho Y323) antibody [EP573-4] (ab62338) at 1/5000 dilution (Diluted with 5% NFDM /TBST)

Lane 1 : Ramos (human Burkitt's lymphoma B lymphocyte) whole cell lysate with 5% NFDM/TBST
Lane 2 : Ramos treated with 1mM pervanadate for 30min whole cell lysate with 5% NFDM/TBST
Lane 3 : Ramos treated with 1mM pervanadate for 30min whole cell lysate. Then the membrane was incubated with alkaline phosphatase. with 5% NFDM/TBST

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 72 kDa



Anti-Syk (phospho Y323) antibody [EP573-4] (ab62338)

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