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Product datasheet

Anti-Syk antibody [SYK-01] ab3993

KO VALIDATED

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Overview

Product name	Anti-Syk antibody [SYK-01]
Description	Mouse monoclonal [SYK-01] to Syk
Host species	Mouse
Specificity	The antibody reacts with Protein tyrosine kinase p72Syk (Syk family tyrosine-specificphospho-transferase).
Tested applications	**Suitable for:** WB, Flow Cyt **Unsuitable for:** ICC
Species reactivity	**Reacts with:** Rat, Human
Immunogen	Recombinant fragment, corresponding to amino acids 5-360 of Human Syk.
Positive control	Flow Cyt: Ramos cells WB: Ramos cell lysate, Rat basophilic leukemia cell lysate, HeLa cell lysate; HEK-293T cell lysate, HAP1 cell lysate.
General notes	The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.097% Sodium azide Constituent: PBS
Purity	Protein A purified
Clonality	Monoclonal

Clone number	SYK-01
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab3993 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	★★★★☆ **(1)**	Use a concentration of 1 - 2 µg/ml.
Flow Cyt		Use 1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

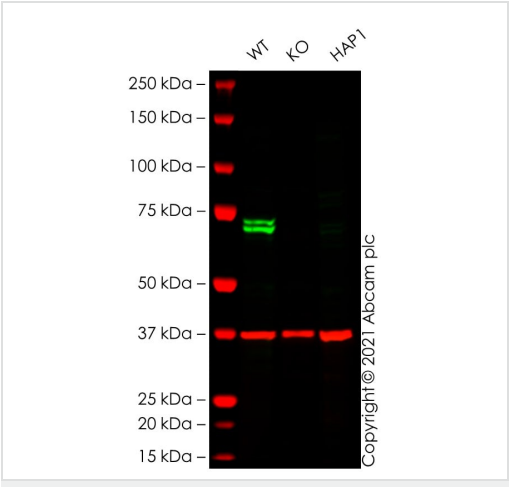
Application notes Is unsuitable for ICC.

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 antibody [SYK-01] (ab3993)

All lanes : Anti-Syk antibody [SYK-01] (ab3993) at 1 µg/ml

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : SYK knockout HEK-293T cell lysate
Lane 3 : HAP1 cell lysate

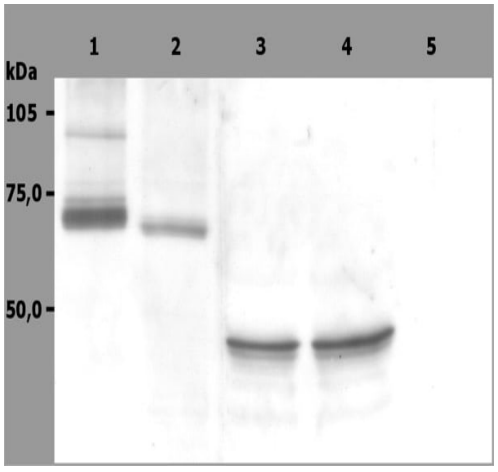
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 72,73 kDa

False colour image of Western blot: Anti-Syk antibody [SYK-01] staining at 1 ug/ml, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (**ab181602**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab3993 was shown to bind specifically to Syk. A band was observed at 72/73 kDa in wild-type

HEK-293T cell lysates with no signal observed at this size in SYK knockout cell line **ab282649** (knockout cell lysate **ab283048**). To generate this image, wild-type and SYK knockout HEK-293T 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) at 1/20000 dilution.



Western blot - Anti-Syk antibody [SYK-01] (ab3993)

Lanes 1-2 : Anti-Syk antibody [SYK-01] (ab3993) at 2 µg/ml
Lanes 3-4 : anti-Human Cytokeratin 18

Lane 1 : Ramos (human Burkitt's lymphoma cell line) cell lysate
Lane 2 : RBL (rat basophilic leukemia cell line) cell lysate
Lanes 3-4 : HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 5 : negative control

Performed under non-reducing conditions.

Flow Cytometry - Anti-Syk antibody [SYK-01] (ab3993)

Overlay histogram showing Ramos (Human Burkitt's lymphoma cell line) cells stained with ab3993 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab3993, 1µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in Ramos cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

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