abcam

ab3993

Anti-Syk antibody [SYK-01]

KO Validated

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.4 Preservative: 0.097% Sodium azide Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Human SYK aa 1-400. The exact immunogen used to generate this antibody is proprietary information. Database link P43405
Clone number	SYK-01
Purification technique	Affinity purification Protein A
Specificity	The antibody reacts with Protein tyrosine kinase p72Syk (Syk family tyrosine-specificphospho-transferase).
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

ICC/IF

Tested

Species	Human
Dilution info	1/1000

Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	1 µg for 10^6 Cells
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Human
Dilution info	1/500
Notes	-

Species	Rat
Dilution info	1-2 g/mL
Notes	-

Target data

See full target information SYK

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 (PubMed:12387735, PubMed:33782605). 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 DEPTOR, VAV1, PLCG1, PI-3-kinase, LCP2 and BLNK (PubMed:12456653, PubMed:15388330, PubMed:34634301, PubMed:8657103). 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 (PubMed:12456653). 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 also plays a role in T-cell receptor signaling. Also plays 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 (By similarity). Required for the stimulation of neutrophil phagocytosis by IL15 (PubMed:15123770). Also mediates the activation of dendritic cells by cell necrosis stimuli. Also involved in mast cells activation. Involved in interleukin-3/IL3-mediated signaling pathway in basophils (By similarity). Also functions downstream of receptors mediating cell adhesion (PubMed:12387735). Relays for instance, integrin-mediated neutrophils and macrophages activation and P-selectin receptor/SELPG-mediated recruitment of leukocytes to inflammatory loci. Also plays 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. Together with CEACAM20, enhances production of the cytokine CXCL8/IL-8 via the NFKB pathway and may thus have a role in the intestinal immune response (By similarity).

Storage

Shipped at conditions	Blue Ice

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

Supplementary info

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

Activity summary	Spleen tyrosine kinase commonly known as Syk is a non-receptor tyrosine kinase with a mass of about 72 kDa. It serves as a critical signaling molecule in immune cells. Syk is expressed in various cell types such as B cells T cells monocytes and others involved in the immune response. It initiates signaling cascades by binding to immunoreceptor tyrosine-based activation motifs (ITAMs). Alternate forms include phosphorylated Syk often referred to as p-Syk which activates various downstream signaling pathways.
Biological function summary	Syk plays a role in transmitting signals from the cell surface to the interior of the cell facilitating various cellular activities. It is a part of signal transduction complexes and functions in the regulation of immune cell activation differentiation and survival. It affects processes like Fc receptor signaling in macrophages and neutrophils impacting immune responses and inflammation. The phospho-Syk form influences the strength and duration of signaling affecting various cellular functions.
Pathways	Syk is significant in the B-cell receptor (BCR) signaling pathway and Fc receptor signaling pathway. Within these pathways Syk interacts with proteins like Lyn and Fyn which are other kinases involved in BCR signal transduction. Syk phosphorylation noted as p-Syk plays a part in activating downstream molecules such as phospholipase C gamma (PLCγ) to mediate cellular responses to external stimuli. The critical role of Syk in these pathways influences both adaptive and innate immune responses.
Associated diseases and disorders	Syk correlates with conditions like rheumatoid arthritis and certain B-cell lymphomas. In rheumatoid arthritis aberrant Syk signaling contributes to the inflammation and joint destruction characteristic of the disease. Syk's role in B-cell antigen receptor signaling links it to B-cell lymphomas where dysregulation in signaling pathways leads to uncontrolled proliferation of malignant B cells. The involvement of Syk in both disease contexts suggests its potential as a therapeutic target along with pathways related to proteins like BLNK and PLCγ in these disorders.

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.

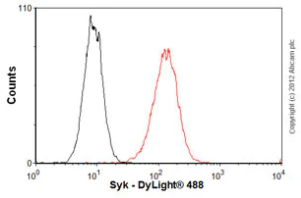
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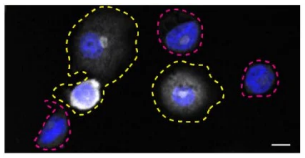
5 product images



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

Syk Flow Cytometry staining using mouse Anti-Syk antibody

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 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. 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.

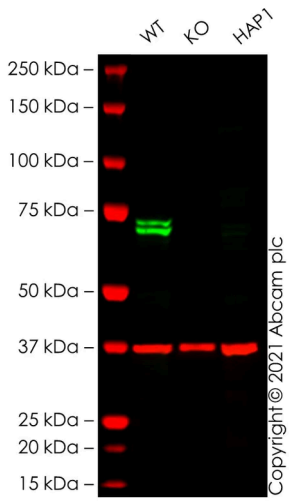


Immunocytochemistry/ Immunofluorescence - Anti-Syk antibody [SYK-01] (ab3993)

ab3993 was shown to react with SYK in wild-type THP-1 cells in immunocytochemistry with loss of signal observed in SYK knockout cell line ab288700. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then

permeabilized with 0.1% Triton X-100 (10min) and then blocked with 1x PBS, 0.01% Triton X-100, 5% BSA, 5% NGS. The cells were then incubated with ab3993 at dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat anti-rabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and far-red (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880).

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



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

Syk Western blot staining using mouse Anti-Syk antibody

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.
All lanes:
Western blot - Anti-Syk antibody [SYK-01] (ab3993) at 1 µg/mL

Lane 1:
Wild-type HEK-293T cell lysate at 20 µg

Lane 2:
SYK knockout HEK-293T cell lysate at 20 µg

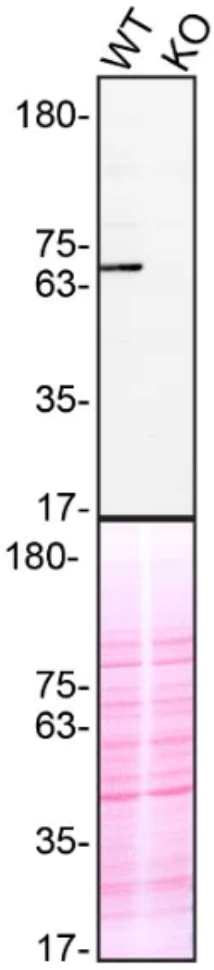
Lane 2:
Western blot - Human SYK knockout HEK-293T cell line (ab282649)

Lane 3:
HAP1 cell lysate at 20 µg

Performed under reducing conditions.

Predicted band size: 72 kDa

Observed band size: 72 kDa, 73 kDa



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

ab3993 was shown to react with SYK in wild-type THP-1 cells in Western blot with loss of signal observed in SYK knockout cell line ab288700. Wild-type THP-1 and SYK knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab3993 overnight at 4 °C at a 1/500 dilution. Blots were incubated with secondary antibodies at 0.2ug/mL before imaging.

This data was provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.
All lanes:
Western blot - Anti-Syk antibody [SYK-01] (ab3993) at 1/500 dilution

Lane 1:
Wild-type THP-1 lysate at 20 µg

Lane 2:
Western blot - Human SYK knockout THP-1 cell line (ab288700) at 20 µg

Observed band size: 72 kDa

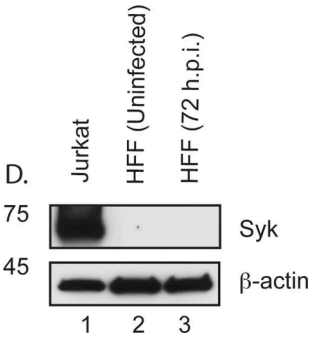


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Western blot - Anti-Syk antibody [SYK-01] (ab3993)

Syk western blot using anti-Syk antibody [SYK-01] ab3993. Publication image and figure legend from Ho, C. M., Donovan-Banfield, I. Z., et al., 2016, PLoS One, PubMed 26930276.

ab3993 was used in this publication in western blot. This may not be the same as the application(s) guaranteed by Abcam. For a full list of applications guaranteed by Abcam for ab3993 please see the product overview.

Investigation of roles for SYK and GCK in HCMV infected cells.(A and B) Analysis of siRNA treated cells infected with AD169. HFF were treated with the siRNAs indicated in each figure for 72 hours and infected with 1x105 plaque forming units (p.f.u.) of HCMV. At 96 h.p.i cell lysates were prepared for western blot (Fig 2A) and viral supernatants were harvested for virus titration (Fig 2B). In Fig 2B viral titre is expressed as plaque forming units/ml (p.f.u./ml) and the mean and standard deviation of 3 experiments is shown. (C) Analysis of HCMV infected cells treated with inhibitors of GCK. HFF cells were infected with AD169 at an MOI of 1 then treated with 1µM of the drug indicated in the figure or the equivalent volume of DMSO. Viral supernatants were harvested at the indicated time points and viral titre (p.f.u./ml) at each time point was determined. (D and E) Western blot of lysate from uninfected or infected cell lines. Unless stated otherwise, all lysates are from HFF cells. Where indicated, HFF cells were either uninfected or infected with AD169 at an MOI of 1 then treated with either 1µM BAY61-3606, 1µM Maribavir or the equivalent volume of DMSO. Cell lysates were prepared for western blot at 72 hours post infection. In each panel showing western blot proteins recognized by the antibodies used in each experiment are indicated to the right of each figure and the positions of molecular weight markers (kDa) are indicated to the left of each figure.

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