abcam

Product datasheet

Anti-Syk antibody [EP573Y] ab40781



19 References 画像数8

製品の概要

製品名 Anti-Syk antibody [EP573Y]

製品の詳細 Rabbit monoclonal [EP573Y] to Syk

由来種 Rabbit

特異性 The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

アプリケーション 適用あり: WB, IHC-P

適用なし: Flow Cyt (Intra) or ICC/IF

種交差性 交差種: Mouse, Human

非交差種: Rat

免疫原 Synthetic peptide within Human Syk aa 300-400. The exact sequence is proprietary.

ポジティブ・コントロール WB: K562, Daudi, WEHI-231 and Raji cell lysates. Human, mouse marrow lysates. IHC-P: Human

spleen.

特記事項 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**.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル **ウローン名** EP573Y **I**gG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab40781の使用に適用されますアプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		1/1000 - 1/5000. Detects a band of approximately 72 kDa (predicted molecular weight: 72 kDa).
IHC-P		1/5000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See IHC antigen retrieval protocols. For unpurified use at 1/100 - 1/250.

追加情報

Is unsuitable for Flow Cyt (Intra) or ICC/IF.

ターゲット情報

機能

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 NFkappa-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 nonimmune 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.

組織特異性

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.

配列類似性

Belongs to the protein kinase superfamily. Tyr protein kinase family. SYK/ZAP-70 subfamily.

Contains 1 protein kinase domain.

Contains 2 SH2 domains.

ドメイン

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.

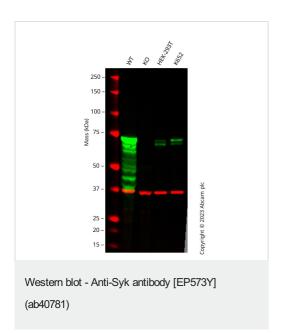
翻訳後修飾

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.

細胞内局在

Cell membrane. Cytoplasm, cytosol.

画像



All lanes : Anti-Syk antibody [EP573Y] (ab40781) at 1/1000 dilution

Lane 1: Wild-type THP-1 cell lysate

Lane 2: SYK knockout THP-1 cell lysate

Lane 3: HEK-293T cell lysate

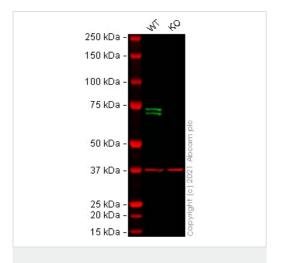
Lane 4: K562 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Western blot: Anti-SYK antibody [EP573Y] (ab40781) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab40781 was shown to bind specifically to SYK. A band was observed at 72 kDa in wild-type THP-1 cell lysates with no signal observed at this size in SYK knockout cell line ab288700 (knockout cell lysate ab289593). To generate this image, wild-type and SYK knockout THP-1 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 5 % 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-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-Syk antibody [EP573Y] (ab40781)

All lanes : Anti-Syk antibody [EP573Y] (ab40781) at 1/1000 dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: SYK knockout HEK-293T cell lysate

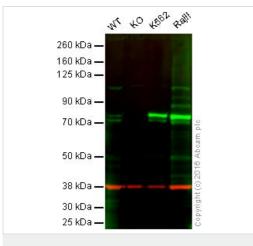
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 72 kDa **Observed band size:** 70 kDa

False colour image of Western blot: Anti-Syk antibody [EP573Y] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab40781 was shown to bind specifically to Syk. A band was observed at 70/72 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-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) at 1/20000 dilution.



Western blot - Anti-Syk antibody [EP573Y] (ab40781)

Lane 1: Wild-type HAP1 cell lysate (40 µg)

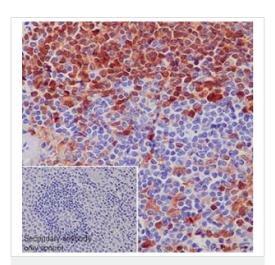
Lane 2: SYK knockout HAP1 cell lysate (40 µg)

Lane 3: K562 cell lysate (40 µg)

Lane 4: Raji cell lysate (40 µg)

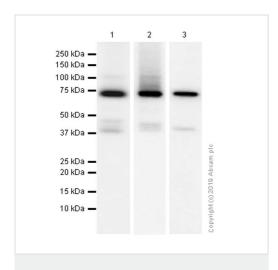
Lanes 1 - 4: Merged signal (red and green). Green - ab40781 observed at 75 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab40781 was shown to specifically react with Syk when Syk knockout samples were used. Wild-type and Syk knockout samples were subjected to SDS-PAGE. Ab40781 and ab8245 (loading control to GAPDH) were diluted at 1/5000 and 1/10000 dilution respectively and incubated overnight at 4C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Syk antibody [EP573Y] (ab40781)

Immunohistochemistry of paraffin embedded Human spleen tissue section labelling Syk with ab40781 at 1:5000 dilution (0.44 μ g/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). A ready to use ImmunoHistoProbe one step HRP Polymer (ready to use) was used as a secondary antibody at 1:0 dilution. PBS instead of primary antibody was used for negative control. Hematoxylin was used as a counterstain.



Western blot - Anti-Syk antibody [EP573Y] (ab40781)

All lanes : Anti-Syk antibody [EP573Y] (ab40781) at 1/1000 dilution (Purified)

Lane 1 : Raji (Human Burkitt's lymphoma B lymphocyte) whole cell lysates

Lane 2 : Daudi (Human Burkitt's lymphoma lymphoblast) whole cell

Lane 3: WEHI-231 (Mouse B cell lymphoma B lymphocyte) whole cell lysates

Lysates/proteins at 15 µg per lane.

Secondary

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

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

Observed band size: 72 kDa

Blocking/Diluting buffer: 5% NFDM/TBST

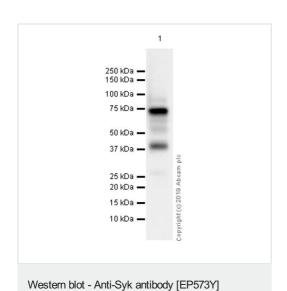
Anti-Syk antibody [EP573Y] (ab40781) at 1/1000 dilution (Purified) + Human bone marrow lysates at 15 μg

Secondary

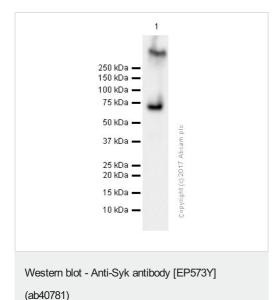
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

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

Blocking/Diluting buffer: 5% NFDM/TBST



(ab40781)



Anti-Syk antibody [EP573Y] (ab40781) at 1/5000 dilution + Mouse bone marrow lysate at 20 µg

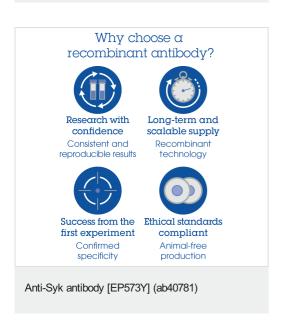
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 72 kDa

Exposure time: 3 minutes

Blocking and diluting buffer: 5% NFDM/TBST.



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