

Human SYK knockout HEK-293T cell line ab282649

3 Images

Overview

Product name	Human SYK knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 127 bp deletion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: WB
Biosafety level	2
General notes	<p>Recommended control: Human wild-type HEK-293T cell line (ab282205). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>Initial handling guidelines: Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability.</p> <ol style="list-style-type: none">1. Thaw the vial in 37°C water bath for approximately 1-2 minutes.2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution.3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of 2×10^4 cells/cm². Seeding density is given as a guide only and should be scaled to align with individual lab schedules.4. Incubate the culture at 37°C incubator with 5% CO₂. Cultures should be monitored daily. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

Cells should be passaged when they have achieved 80-90% confluence.

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We will provide viable cells that proliferate on revival.

Properties

Number of cells	1 x 10 ⁶ cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Kidney
Cell type	epithelial
Gender	Female
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

Target

Function	<p>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</p>
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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.

Applications

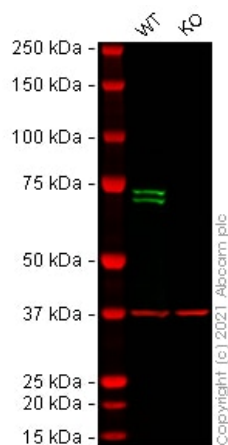
The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab282649 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 72,73 kDa (predicted molecular weight: 72 kDa).

Images



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

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

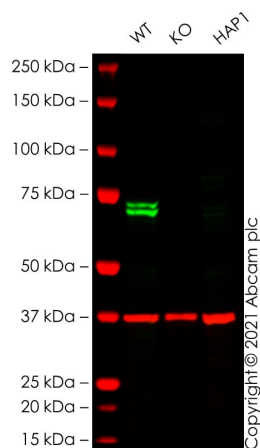
Lysates/proteins at 20 µg per lane.

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 - Human SYK knockout HEK-293T cell line (ab282649)

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.

Predicted band size: 72 kDa

Observed band size: 72,73 kDa

False colour image of Western blot: Anti-Syk antibody [SYK-01] staining at 1 µg/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.

127 bp deletion in exon 2

G4 - 127 bp deletion (Main clone)

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G4  GCACATCACC-----
      |||||
WT  GCACATCACC0GGGAGGAGCAGAGATTACTGCTCAGGGGGCATGAGTGTGGCTTTATTGCTGGCC

G4  -----CACCATCGAGC
      |||||
WT  AGAGCGCACTACTGAGTGGCTTGGCCCTGTCCGTGGCCACGGGAGGAGGACACCACTACACCATCGAGC
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Sanger Sequencing - Human SYK knockout HEK-293T cell line (ab282649)

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