abcam

Product datasheet

Human FYN knockout HEK-293 cell line ab269630

3 Images

Overview

Product name	Human FYN knockout HEK-293 cell line
Parental Cell Line	HEK-293
Organism	Human
Mutation description	Knockout achieved by CRISPR/Cas9; X = 14 bp deletion; Frameshift: 100%
Passage number	<20
Knockout validation	Next Generation Sequencing (NGS), Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HEK-293 cell line (**ab259776**). 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. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** DMEM (High Glucose) + 10% FBS **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. 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 $2x10^4$ cells/cm^2. 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% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.

Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

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	Tyrosine-protein kinase implicated in the control of cell growth. Plays a role in the regulation of intracellular calcium levels, with isoform 2 showing the greater ability to mobilize cytoplasmic calcium in comparison to isoform 1. Required in brain development and mature brain function with important roles in the regulation of axon growth, axon guidance, and neurite extension. Blocks axon outgrowth and attraction induced by NTN1 by phosphorylating its receptor DDC. Phosphorylates RUNX3.
Tissue specificity	Isoform 1 is highly expressed in the brain. Isoform 2 is expressed in cells of hemopoietic lineages, especially T lymphocytes.
Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. SRC subfamily. Contains 1 protein kinase domain. Contains 1 SH2 domain. Contains 1 SH3 domain.
Cellular localization	Cell membrane. Present and active in lipid rafts. Present in cell body and along the process of mature and developing oligodendroyctes.
Form	This protein is known to be similar in amino acid sequence to HCK (P08631), LCK (P06239), YES1 (P07947), SRC (P12931), and LYN (P07948). Therefore, cross-reactivity with these homologous proteins may be observed. We would be happy to provide immunogen alignment information upon request.

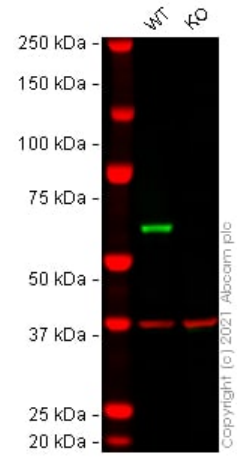
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab269630 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. Predicted molecular weight: 60 kDa.

Images



Western blot - Human FYN knockout HEK-293 cell line (ab269630)

All lanes : Anti-Fyn antibody [EPR19636] (**ab184276**) at 1/1000 dilution

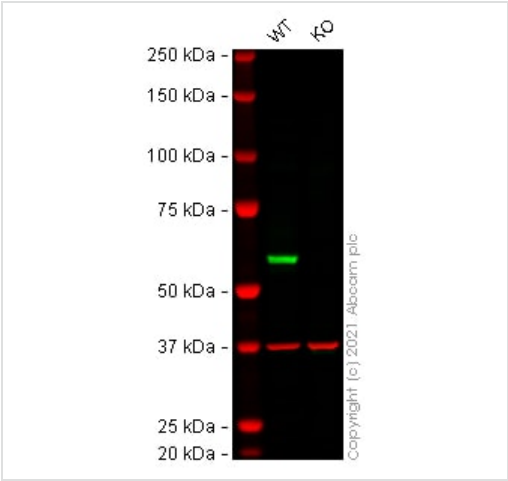
Lane 1 : Wild-type HEK-293 cell lysate
Lane 2 : FYN knockout HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 60 kDa
Observed band size: 60 kDa

False colour image of Western blot: Anti-Fyn antibody [EPR19636] 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, **ab184276** was shown to bind specifically to Fyn. A band was observed at 60 kDa in wild-type HEK-293 cell lysates with no signal observed at this size in FYN knockout cell line ab269630 (knockout cell lysate **ab272440**). To generate this image, wild-type and FYN knockout HEK-293 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 FYN knockout HEK-293 cell line (ab269630)

All lanes : Anti-Fyn antibody [EPR5500] (**ab125016**) at 1/1000 dilution

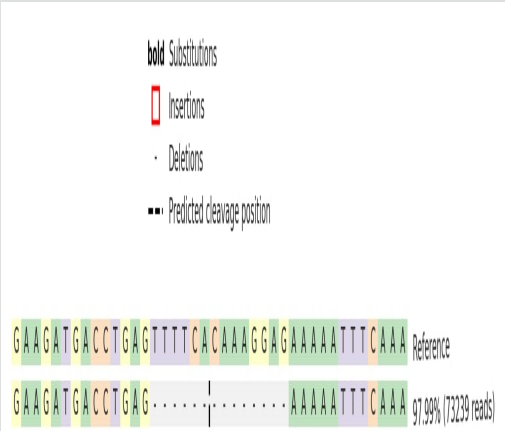
Lane 1 : Wild-type HEK-293 cell lysate
Lane 2 : FYN knockout HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 60 kDa
Observed band size: 60 kDa

False colour image of Western blot: Anti-Fyn antibody [EPR5500] 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, **ab125016** was shown to bind specifically to Fyn. A band was observed at 60 kDa in wild-type HEK-293 cell lysates with no signal observed at this size in FYN knockout cell line ab269630 (knockout cell lysate **ab272440**). To generate this image, wild-type and FYN knockout HEK-293 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.



Next Generation Sequencing - Human FYN knockout HEK-293 cell line (ab269630)

Knockout achieved by CRISPR/Cas9; X = 14 bp deletion;
Frameshift: 100%

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