abcam

Product datasheet

Human FYN knockout HEK-293T cell lysate ab257071

2 Images

Overview

Product name	Human FYN knockout HEK-293T cell lysate	
Product overview	Knockey to all bracts askinged by CDICDD/Case	
	Knockout cell lysate achieved by CRISPR/Cas9.	
Parental Cell Line	HEK293T	
Organism	Human	
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 4.	
Passage number	<20	
Knockout validation	Sanger Sequencing, Western Blot (WB)	
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. *Usage of SDS sample buffer is not recommended with these lyophilized lysates.	
Notes	Lysate preparation: Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). <i>This means that the protein of interest is denatured.</i> If you require a native form of the protein please use the live cell version - found <u>here</u> . Please refer to our lysis protocol for further details on how our lysates are prepared.	
	User storage instructions: Lyophilizate may be stored at 4°C. After reconstitution, store at - 20°C for short-term storage or -80°C for long-term storage.	
	Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. See here for more information on knockout cell lysates.	
	Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.	
	This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our <u>limited use license</u> and <u>patent pages</u> .	
Tested applications	Suitable for: WB	

Properties

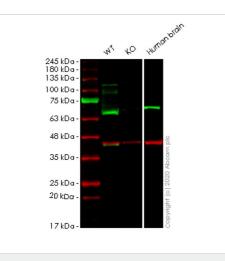
Storage instructions	Store at -80°C. Please refer to protocols.		
Components		1 kit	
ab260925 - Human FYN knockout HEK293T cell lysate		1 x 100µg	
ab255553 - Human wild-type HEK293T cell lysate		1 x 100µg	
Cell type	epithelial		
STR Analysis	Amelogenin X D5S818: 8, 9 D13S317: 12, 14 D7S820: 11 D16S539: 9, 13 vWA: 16, 19 TH01: 7, 9.3 TPOX: 11 CSF1PO: 11, 12		
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	lsoform 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 YES1 (P07947), SRC (P12931), and LYN (P07948). Therefore homologous proteins may be observed. We would be happy information upon request.	ore, cross-reactivity with these	

Applications

The Abpromise guarantee	Our Abpromise guarantee covers the use of ab257071 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.



Western blot - Human FYN knockout HEK293T cell Iysate (ab257071)

AAT GT AAGGAT AAAGAAGCAACAAAACT GAACGGAGGAGGGGACGGCAGCCT GAACCAG

AATGTAAGGATAAAGAAGCAACAAAACTGA CGGAGGAGGGGACGGCAGCCTGAACCAG

Sanger Sequencing - Human FYN knockout

HEK293T cell lysate (ab257071)

Mut

WT

Lane 1: Wild-type HEK293T cell lysate (20 µg)

Lane 2: FYN knockout HEK293T cell lysate (20 µg)

Lane 3: Human brain tissue lysate (20 µg)

Lanes 1-3: Merged signal (red and green). Green - <u>ab184276</u> observed at 60 kDa. Red - loading control, <u>ab8245</u> observed at 37 kDa.

ab184276 Anti-Fyn antibody [EPR19636] was shown to specifically react with Fyn in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab266133** (knockout cell lysate ab257071) was used. Wild-type and Fyn knockout samples were subjected to SDS-PAGE. **ab184276** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. 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 in 10000 dilution for 1 hour at room temperature before imaging.

Homozygous: 1 bp insertion in exon 4

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