abcam

Product datasheet

Human PTK2 (FAK) knockout HEK-293T cell line ab255421

4 Images

Overview

Product name	Human PTK2 (FAK) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 16 bp deletion in exon 4 and 17 bp deletion in exon 4
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**Recommended control:** Human wild-type HEK293T cell line (**ab255449**). 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
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
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	Non-receptor protein-tyrosine kinase implicated in signaling pathways involved in cell motility, proliferation and apoptosis. Activated by tyrosine-phosphorylation in response to either integrin clustering induced by cell adhesion or antibody cross-linking, or via G-protein coupled receptor (GPCR) occupancy by ligands such as bombesin or lysophosphatidic acid, or via LDL receptor occupancy. Microtubule-induced dephosphorylation at Tyr-397 is crucial for the induction of focal adhesion disassembly. Plays a potential role in oncogenic transformations resulting in increased kinase activity.
Tissue specificity	Expressed in all organs tested, in lymphoid cell lines, but most abundantly in brain.
Sequence similarities	Belongs to the protein kinase superfamily. Tyr protein kinase family. FAK subfamily. Contains 1 FERM domain. Contains 1 protein kinase domain.
Domain	The first Pro-rich domain interacts with the SH3 domain of CRK-associated substrate (BCAR1) and CASL. The carboxy-terminal region is the site of focal adhesion targeting (FAT) sequence which mediates the localization of FAK1 to focal adhesions.
Post-translational modifications	Phosphorylated on 6 tyrosine residues upon activation. Microtubule-induced dephosphorylation at Tyr-397 could be catalyzed by PTPN11 and regulated by ZFYVE21. Dephosphorylated by PTPN11 upon EPHA2 activation by its ligand EFNA1.
Cellular localization	Cell junction > focal adhesion. Cell membrane. Constituent of focal adhesions.

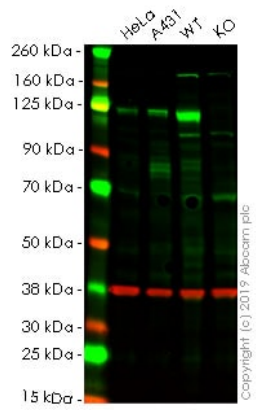
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab255421 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: 119 kDa.

Images



Western blot - Human PTK2 (FAK) knockout HEK-293T cell line (ab255421)

All lanes : Anti-FAK antibody [EP1831Y] (**ab76496**) at 1/500 dilution

Lane 1 : HeLa cell lysate
Lane 2 : A431 cell lysate
Lane 3 : Wild-type HEK-293T cell lysate
Lane 4 : PTK2 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

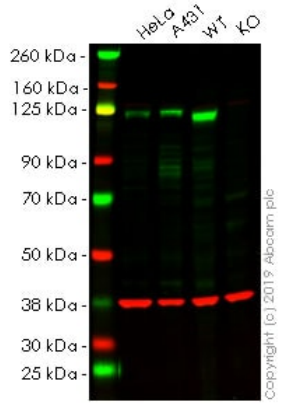
Performed under reducing conditions.

Predicted band size: 119 kDa
Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - **ab76496** observed at 119 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab76496 was shown to react with FAK in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab255421 (knockout cell lysate **ab263766**) was used. Wild-type and FAK knockout samples were subjected to SDS-PAGE. **ab76496** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 500 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 20000 dilution for 1 hour

at room temperature before imaging.



Western blot - Human PTK2 (FAK) knockout HEK-293T cell line (ab255421)

All lanes : Anti-FAK antibody [EP695Y] (**ab40794**) at 1/1000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : A431 cell lysate
Lane 3 : Wild-type HEK-293T cell lysate
Lane 4 : PTK2 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

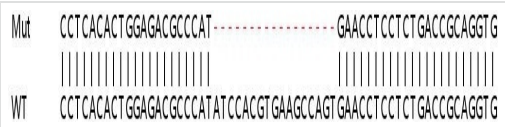
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 119 kDa
Additional bands at: 37 kDa (possible Loading Control)

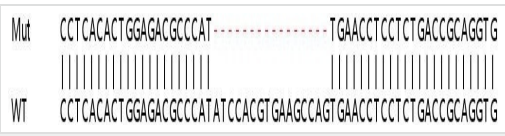
Lanes 1 - 4: Merged signal (red and green). Green - **ab40794** observed at 119 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab40794 was shown to react with FAK in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line ab255421 (knockout cell lysate **ab263766**) was used. Wild-type and FAK knockout samples were subjected to SDS-PAGE. **ab40794** 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 20000 dilution for 1 hour at room temperature before imaging.



Sanger Sequencing - Human PTK2 knockout HEK293T cell line (ab255421)

Allele-1: 17 bp deletion in exon 4



Sanger Sequencing - Human PTK2 knockout HEK293T cell line (ab255421)

Allele-2: 16 bp deletion in exon 4.

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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