abcam

Product datasheet

Human ZYX (Zyxin) knockout HEK-293T cell line ab266504

4 Images

Overview

Product name	Human ZYX (Zyxin) knockout HEK-293T cell line
Parental Cell Line	HEK293T
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: Insertion of the selection cassette in exon 2
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, 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 **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	Adhesion plaque protein. Binds alpha-actinin and the CRP protein. Important for targeting TES and ENA/VASP family members to focal adhesions and for the formation of actin-rich structures. May be a component of a signal transduction pathway that mediates adhesion-stimulated changes in gene expression.
Sequence similarities	Belongs to the zyxin/ajuba family. Contains 3 LIM zinc-binding domains.
Cellular localization	Cytoplasm. Cytoplasm, cytoskeleton. Nucleus. Cell junction, focal adhesion. Associates with the actin cytoskeleton near the adhesion plaques. Enters the nucleus in the presence of HESX1.

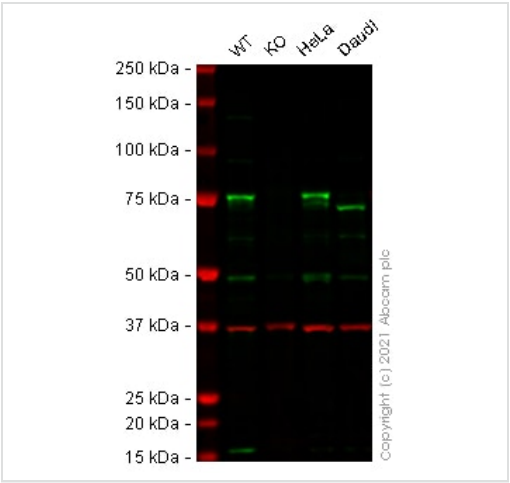
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab266504 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: 61 kDa.

Images



Western blot - Human ZYX (Zyxin) knockout HEK-293T cell line (ab266504)

All lanes : Anti-Zyxin antibody - N-terminal (**ab229757**) at 1/1000 dilution

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

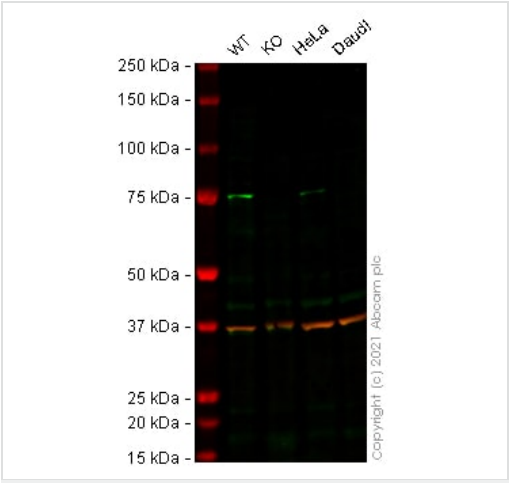
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 61 kDa
Observed band size: 75 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab229757** observed at 75 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab229757 was shown to react with ZYX in wild-type HEK-293T cells in Western blot with loss of signal observed in ZYX knockout cell line ab266504 (ZYX knockout cell lysate **ab257810**). Wild-type HEK-293T and ZYX knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab229757** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human ZYX (Zyxin) knockout HEK-293T cell line (ab266504)

All lanes : Anti-Zyxin antibody [ZOL301] (**ab50391**) at 1/1000 dilution

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

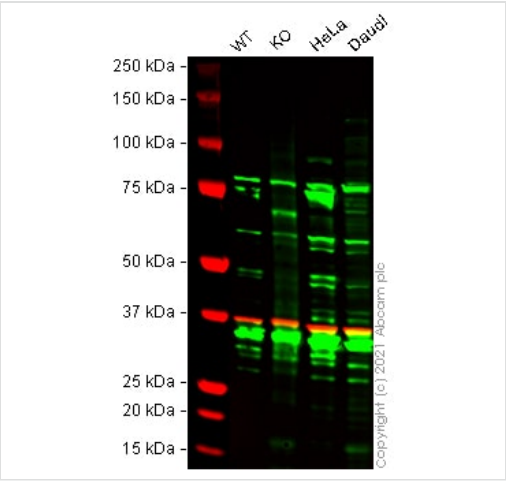
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 61 kDa
Observed band size: 75 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab50391** observed at 75 kDa. Red - loading control **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) observed at 37 kDa.

ab50391 was shown to react with ZYX in wild-type HEK-293T cells in Western blot with loss of signal observed in ZYX knockout cell line ab266504 (ZYX knockout cell lysate **ab257810**). Wild-type HEK-293T and ZYX knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab50391** and **ab181602** (Rabbit Anti-GAPDH antibody [EPR16891]) overnight at 4 °C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Western blot - Human ZYX (Zyxin) knockout HEK-293T cell line (ab266504)

All lanes : Anti-Zyxin antibody [EPR4302] (**ab109316**) at 1/20000 dilution

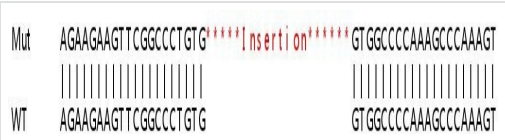
Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : ZYX knockout HEK-293T cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : Daudi cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 61 kDa
Observed band size: 75 kDa

False colour image of Western blot: Anti-Zyxin antibody [EPR4302] staining at 1/20000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab109316** was shown to bind specifically to Zyxin. A band was observed at 75 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in ZYX knockout cell line ab266504 (knockout cell lysate **ab257810**). The band observed in the knockout lysate lane below 75 kDa is likely to represent a truncated form of Zyxin. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and ZYX 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 fluorescent western blot (TBS-based) blocking solution 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.



Sanger Sequencing - Human ZYX knockout HEK293T cell line (ab266504)

Homozygous: Insertion of the selection cassette in exon 2

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