abcam

Product datasheet

Human ACTN1 (alpha Actinin) knockout HeLa cell line ab265610

2 Images

Overview

Product name Human ACTN1 (alpha Actinin) knockout HeLa cell line

Parental Cell Line HeLa
Organism Human

Mutation description Knockout achieved by using CRISPR/Cas9, Homozygous: 4 bp deletion in exon 2

Passage number <20

Knockout validation Sanger Sequencing, Western Blot (WB)

Tested applications Suitable for: WB

Biosafety level 2

General notesRecommended control: Human wild-type HeLa cell line (<u>ab255928</u>). 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 2x10⁴ 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.

Subculture guidelines:

All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm² is recommended.

1

A partial media change 24 hours prior to subculture may be helpful to encourage growth, if

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 10⁶ cells/vial, 1 mL

Adherent /Suspension Adherent
Tissue Cervix
Cell type epithelial

Disease Adenocarcinoma

Gender Female

STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18

TH01: 7 TPOX: 8,12 CSF1PO: 9, 10

Antibiotic resistance Puromycin 1.00µg/ml

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 F-actin cross-linking protein which is thought to anchor actin to a variety of intracellular structures.

This is a bundling protein.

Sequence similaritiesBelongs to the alpha-actinin family.

Contains 1 actin-binding domain.

Contains 2 CH (calponin-homology) domains.

Contains 2 EF-hand domains. Contains 4 spectrin repeats.

Cellular localization Cytoplasm > cytoskeleton. Cytoplasm > myofibril > sarcomere > Z line. Colocalizes with MYOZ2

and PPP3CA at the Z-line of heart and skeletal muscle.

Applications

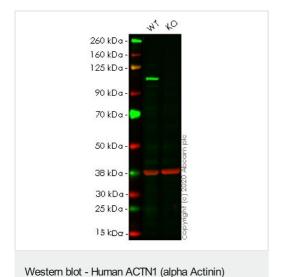
The Abpromise guarantee Our Abpromise guarantee covers the use of ab265610 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 103 kDa.

Images



knockout HeLa cell line (ab265610)

All lanes: Anti-alpha Actinin/ACTN1 antibody [EP2527Y] (ab68194) at 1/2000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: ACTN1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 103 kDa

Observed band size: 103 kDa

Lanes 1-2: Merged signal (red and green). Green - ab68194 observed at 103 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (ab8245) observed at 37 kDa.

ab68194 was shown to react with alpha Actinin/ACTN1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265610 (knockout cell lysate ab257337) was used. Wild-type HeLa and ACTN1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab68194 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated overnight at 4°C at a 1 in 2000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG 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.

Mut	CATTCACGGCATGGTGTAACTCCCACGGAAGGCGGGGACACAGATCGAGAACATCG			
WT	${\tt CATTCACGGCATGGTGTAACTCCCACCTCCGGAAGGCGGGGACACAGATCGAGAACATCG}$			
Sanger Sequencing - Human ACTN1 knockout HeLa				

cell line (ab265610)

Homozygous: 4 bp deletion in exon 2.

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