abcam

Product datasheet

Human DIAPH1 knockout HCT116 cell line ab273727

2 Images

Overview

Product name	Human DIAPH1 knockout HCT116 cell line
Parental Cell Line	HCT116
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 2 bp deletion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Recommended control:** Human wild-type HCT116 cell line (**ab273730**). 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:** McCoY5a + 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	Colon
Cell type	epithelial
Disease	Carcinoma
Gender	Male
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	Acts in a Rho-dependent manner to recruit PFY1 to the membrane. Required for the assembly of F-actin structures, such as actin cables and stress fibers. Nucleates actin filaments. Binds to the barbed end of the actin filament and slows down actin polymerization and depolymerization. Required for cytokinesis, and transcriptional activation of the serum response factor. DFR proteins couple Rho and Src tyrosine kinase during signaling and the regulation of actin dynamics. Functions as a scaffold protein for MAPRE1 and APC to stabilize microtubules and promote cell migration (By similarity). Has neurite outgrowth promoting activity (By similarity). In hear cells, it may play a role in the regulation of actin polymerization in hair cells. The MEMO1-RHOA-DIAPH1 signaling pathway plays an important role in ERBB2-dependent stabilization of microtubules at the cell cortex. It controls the localization of APC and CLASP2 to the cell membrane, via the regulation of GSK3B activity. In turn, membrane-bound APC allows the localization of the MACF1 to the cell membrane, which is required for microtubule capture and stabilization.
Tissue specificity	Expressed in brain, heart, placenta, lung, kidney, pancreas, liver, skeletal muscle and cochlea.
Involvement in disease	Defects in DIAPH1 are the cause of deafness autosomal dominant type 1 (DFNA1) [MIM:124900]. DFNA1 is a form of sensorineural hearing loss. Sensorineural deafness results from damage to the neural receptors of the inner ear, the nerve pathways to the brain, or the area of the brain that receives sound information.
Sequence similarities	Belongs to the formin homology family. Diaphanous subfamily. Contains 1 DAD (diaphanous autoregulatory) domain. Contains 1 FH1 (formin homology 1) domain. Contains 1 FH2 (formin homology 2) domain. Contains 1 GBD/FH3 (Rho GTPase-binding/formin homology 3) domain.

Domain	DRFs are regulated by intramolecular GBD-DAD binding where Rho-GTP activates the DRFs by disrupting the GBD-DAD interaction (By similarity). DCAF7 binds to the FH2 (formin homology 2) domain.
Cellular localization	Cell membrane. Cell projection > ruffle membrane. Cytoplasm > cytoskeleton. Membrane ruffles, especially at the tip of ruffles, of motile cells.

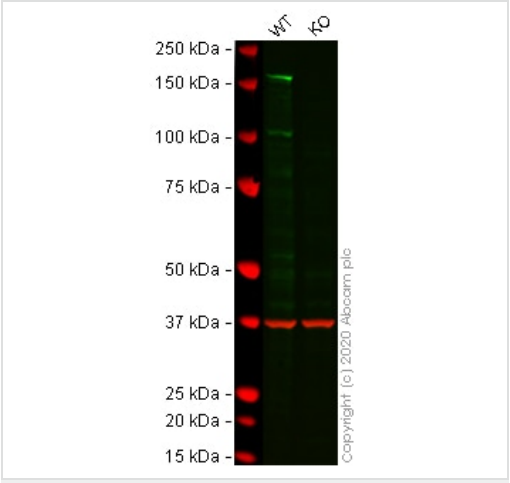
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab273727 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: 141 kDa.

Images



Western blot - Human DIAPH1 knockout HCT116 cell line (ab273727)

All lanes : Anti-DIAPH1 antibody [EPR7948] (**ab129167**) at 1/1000 dilution

Lane 1 : Wild-type HCT116 cell lysate
Lane 2 : DIAPH1 knockout HCT116 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 141 kDa
Observed band size: 155 kDa

Lanes 1 - 2: Merged signal (red and green). Green - **ab129167** observed at 155 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab129167 was shown to react with DIAPH1 in wild-type HCT 116 cells in western blot with loss of signal observed in DIAPH1 knockout cell line ab273727 (DIAPH1 knockout cell lysate **ab275252**). Wild-type and DIAPH1 knockout HCT 116 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab129167** 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 hour at room temperature before imaging.

Sanger Sequencing - Human DIAPH1 knockout HCT116 cell line (ab273727)

Allele-1: 2 bp deletion in exon 1 .

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