abcam

Product datasheet

Human PKP3 (Plakophilin 3) knockout HeLa cell line ab265539

画像数 2

製品の概要

製品名	Human PKP3 (Plakophilin 3) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 5 bp deletion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Biosafety level	2
特記事項	**Recommended control:** Human wild-type HeLa cell line (**ab255928**). 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.

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We will provide viable cells that proliferate on revival.

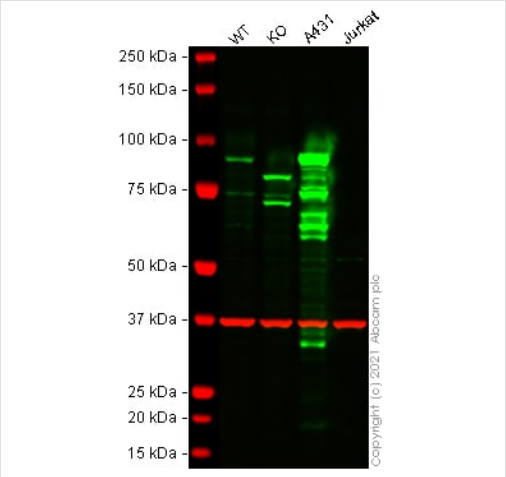
製品の特性

Number of cells	1 x 10^6 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
Mycoplasma free	Yes
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	May play a role in junctional plaques.
組織特異性	Found in desmosomes of most simple and stratified epithelia. Not found in foreskin fibroblasts and various sarcoma-derived cell lines. Beside dendritic reticular cells of lymphatic follicles not found in non-epithelial desmosome-bearing tissues.
配列類似性	Belongs to the beta-catenin family. Contains 8 ARM repeats.
細胞内局在	Nucleus. Cell junction > desmosome. Nuclear and associated with desmosomes.

画像



Western blot - Human PKP3 (Plakophilin 3) knockout HeLa cell line (ab265539)

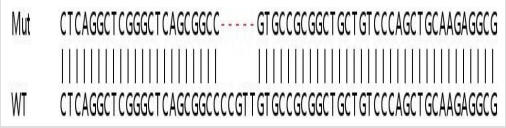
All lanes : Anti-Plakophilin 3 antibody [EPR5560] (**ab109441**) at 1/10000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : PKP3 CRISPR-Cas9 edited HeLa cell lysate
Lane 3 : A431 cell lysate
Lane 4 : Jurkat cell lysate

Lysates/proteins at 20 µg per lane.

Observed band size: 70, 80 kDa

False colour image of Western blot: Anti-Plakophilin 3 antibody [EPR5560] staining at 1/10000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab109441** was shown to bind specifically to Plakophilin 3. A band was observed at 70 and 80 kDa in wild-type HeLa cell lysates with no signal observed at this size in PKP3 CRISPR-Cas9 edited cell line ab265539 (CRISPR-Cas9 edited cell lysate **ab258120**). The band observed in the CRISPR-Cas9 edited lysate lane below 70 and 80 kDa is likely to represent a truncated form of Plakophilin 3. 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 PKP3 CRISPR-Cas9 edited HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) 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 PKP3 knockout HeLa cell line (ab265539)

Homozygous: 5 bp deletion in exon 1.

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