abcam

Product datasheet

Human HPDL knockout HeLa cell line ab265868

3 Images

Overview

Product name	Human HPDL knockout HeLa cell line		
Parental Cell Line	HeLa		
Organism	Human		
Mutation description	Human Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 1 and 2 bp deletion in exon 1		
Passage number			
Knockout validation	<20		
	Sanger Sequencing, Western Blot (WB)		
Tested applications Biosafety level	Suitable for: WB		
General notes	 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.		
	 Thaw the vial in 37°C water bath for approximately 1-2 minutes. 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. 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% 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	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		
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.		
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether		

Target	
Function	May have dioxygenase activity.
Sequence similarities	Belongs to the 4HPPD family.

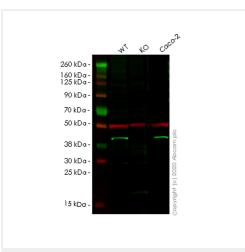
Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab265868 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: 39 kDa.

Images



Western blot - Human HPDL knockout HeLa cell line (ab265868)

All lanes : Anti-HPDL antibody [EPR11691] - C-terminal (<u>ab174841</u>) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate Lane 2 : HPDL knockout HeLa cell lysate Lane 3 : Caco-2 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Predicted band size: 39 kDa Observed band size: 39 kDa

Lanes 1-3: Merged signal (red and green). Green - <u>ab174841</u> observed at 39 kDa. Red - loading control <u>ab7291</u> observed at 50 kDa.

<u>ab174841</u> Anti-HPDL antibody [EPR11691] - C-terminal was shown to specifically react with HPDL in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265868 (knockout cell lysate <u>ab257995</u>) was used. Wild-type and HPDL knockout samples were subjected to SDS-PAGE. <u>ab174841</u> and Anti-alpha Tubulin antibody [DM1A] - Loading Control (<u>ab7291</u>) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye[®] 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye[®] 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Allele-1: 2 bp deletion in exon 1.

Allele-2: 1 bp insertion in exon 1.

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