abcam

Product datasheet

Human TMUB1 partial knockout HeLa cell line ab265852

4 Images

Overview

Product name	Human TMUB1 partial knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp deletion in exon 2 and 1 bp insertion in exon 2
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB, Sanger Sequencing
Biosafety level	2
General notes	**Knockout profile:** Only the long form of the protein (lHOPS, 27 kDa) has been knocked-out from the parental cell line. The band observed in the KO lysate at 21 kDa is likely to represent a short form of the protein (sHOPS) (doi: 10.4161/cc.27054). We have not investigated the function of the remaining form of the protein. **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.

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 contribute to the regulation of translation during cell-cycle progression. May contribute to the regulation of cell proliferation.
Sequence similarities	Contains 1 ubiquitin-like domain.
Cellular localization	Membrane. Cytoplasm. Nucleus. Predominantly nuclear during growth arrest (By similarity). Actively exported from the nucleus in dividing cells.

Applications

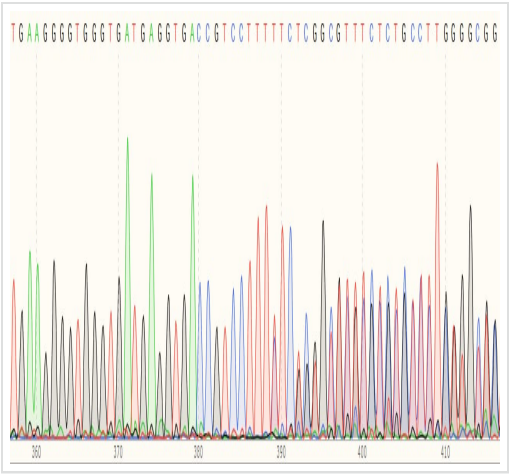
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab265852 in the following tested applications.

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: 26 kDa.

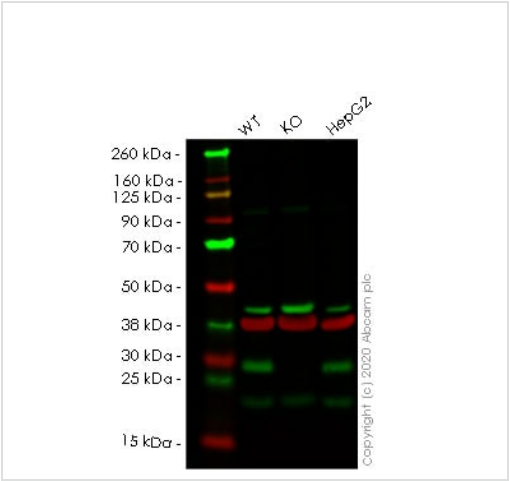
Application	Abreviews	Notes
Sanger Sequencing		Use at an assay dependent concentration.

Images



Sanger Sequencing - Human TMUB1 partial knockout HeLa cell line (ab265852)

Sequencing chromatogram displaying sequence edit in exon 2



Western blot - Human TMUB1 knockout HeLa cell line (ab265852)

All lanes : Anti-TMUB1 antibody [EPR14066] (**ab180586**) at 1/10000 dilution

Lane 1 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 2 : TMUB1 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate
Lane 3 : HepG2 (Human liver hepatocellular carcinoma cell line) cell lysate cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 26 kDa
Observed band size: 27 kDa

Lanes 1- 3: Merged signal (red and green). Green - **ab180586** observed at 27 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab180586 was shown to react with TMUB1 in wild-type HeLa (Human epithelial line from cervix adenocarcinoma) cells in western blot. Loss of signal was observed when knockout cell line ab265852 (knockout cell lysate **ab258237**) was used. Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) and TMUB1 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) 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. **ab180586** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 10000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG 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.

Sanger Sequencing - Human TMUB1 knockout HeLa cell line (ab265852)

Allele-1: 1 bp deletion in exon 2.

Sanger Sequencing - Human TMUB1 knockout HeLa cell line (ab265852)

Allele-2: 1 bp insertion in exon 2.

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