

Human MUC1 knockout HeLa cell line ab255412

2 Images

Overview

Product name	Human MUC1 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 1
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	Suitable for: Sanger Sequencing, WB
Biosafety level	2
General notes	<p>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.</p> <p>Cryopreservation cell medium: Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p>Culture medium: DMEM (High Glucose) + 10% FBS</p> <p>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.</p> <ol style="list-style-type: none">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 2×10^4 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. <p>Subculture guidelines:</p> <p>All seeding densities should be based on cell counts gained by established methods. A guide seeding density of 2×10^4 cells/cm² is recommended.</p> <p>A partial media change 24 hours prior to subculture may be helpful to encourage growth, if required.</p>

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 WWA: 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	<p>The alpha subunit has cell adhesive properties. Can act both as an adhesion and an anti-adhesion protein. May provide a protective layer on epithelial cells against bacterial and enzyme attack.</p> <p>The beta subunit contains a C-terminal domain which is involved in cell signaling, through phosphorylations and protein-protein interactions. Modulates signaling in ERK, SRC and NF-kappa-B pathways. In activated T-cells, influences directly or indirectly the Ras/MAPK pathway. Promotes tumor progression. Regulates TP53-mediated transcription and determines cell fate in the genotoxic stress response. Binds, together with KLF4, the PE21 promoter element of TP53 and represses TP53 activity.</p>
Tissue specificity	Expressed on the apical surface of epithelial cells, especially of airway passages, breast and uterus. Also expressed in activated and unactivated T-cells. Overexpressed in epithelial tumors, such as breast or ovarian cancer and also in non-epithelial tumor cells. Isoform Y is expressed in tumor cells only.
Involvement in disease	<p>MUC1/CA 15-3 is used as a serological clinical marker of breast cancer to monitor response to breast cancer treatment and disease recurrence (PubMed:20816948). Decreased levels over time may be indicative of a positive response to treatment. Conversely, increased levels may indicate disease progression. At an early stage disease, only 21% of patients exhibit high MUC1/CA 15-3 levels, that is why CA 15-3 is not a useful screening test. Most antibodies target the highly immunodominant core peptide domain of 20 amino acid (APDTRPAPGSTAPPAHGVT) tandem repeats. Some antibodies recognize glycosylated epitopes.</p> <p>Medullary cystic kidney disease 1</p>
Sequence similarities	Contains 1 SEA domain.

Developmental stage

During fetal development, expressed at low levels in the colonic epithelium from 13 weeks of gestation.

Post-translational modifications

Highly glycosylated (N- and O-linked carbohydrates and sialic acid). O-glycosylated to a varying degree on serine and threonine residues within each tandem repeat, ranging from mono- to penta-glycosylation. The average density ranges from about 50% in human milk to over 90% in T47D breast cancer cells. Further sialylation occurs during recycling. Membrane-shed glycoproteins from kidney and breast cancer cells have preferentially sialylated core 1 structures, while secreted forms from the same tissues display mainly core 2 structures. The O-glycosylated content is overlapping in both these tissues with terminal fucose and galactose, 2- and 3-linked galactose, 3- and 3,6-linked GalNAc-ol and 4-linked GlcNAc predominating. Differentially O-glycosylated in breast carcinomas with 3,4-linked GlcNAc. N-glycosylation consists of high-mannose, acidic complex-type and hybrid glycans in the secreted form MUC1/SEC, and neutral complex-type in the transmembrane form, MUC1/TM.

Proteolytic cleavage in the SEA domain occurs in the endoplasmic reticulum by an autoproteolytic mechanism and requires the full-length SEA domain as well as requiring a Ser, Thr or Cys residue at the P + 1 site. Cleavage at this site also occurs on isoform MUC1/X but not on isoform MUC1/Y. Ectodomain shedding is mediated by ADAM17.

Dual palmitoylation on cysteine residues in the CQC motif is required for recycling from endosomes back to the plasma membrane.

Phosphorylated on tyrosines and serine residues in the C-terminal. Phosphorylation on tyrosines in the C-terminal increases the nuclear location of MUC1 and beta-catenin. Phosphorylation by PKC delta induces binding of MUC1 to beta-catenin/CTNNB1 and thus decreases the formation of the beta-catenin/E-cadherin complex. Src-mediated phosphorylation inhibits interaction with GSK3B. Src- and EGFR-mediated phosphorylation on Tyr-1229 increases binding to beta-catenin/CTNNB1. GSK3B-mediated phosphorylation on Ser-1227 decreases this interaction but restores the formation of the beta-cadherin/E-cadherin complex. On T-cell receptor activation, phosphorylated by LCK. PDGFR-mediated phosphorylation increases nuclear colocalization of MUC1CT and CTNNB1.

The N-terminal sequence has been shown to begin at position 24 or 28.

Cellular localization

Secreted; Cell membrane. Cytoplasm. Nucleus. On EGF and PDGFRB stimulation, transported to the nucleus through interaction with CTNNB1, a process which is stimulated by phosphorylation. On HRG stimulation, colocalizes with JUP/gamma-catenin at the nucleus and Apical cell membrane. Exclusively located in the apical domain of the plasma membrane of highly polarized epithelial cells. After endocytosis, internalized and recycled to the cell membrane. Located to microvilli and to the tips of long filopodial protrusions.

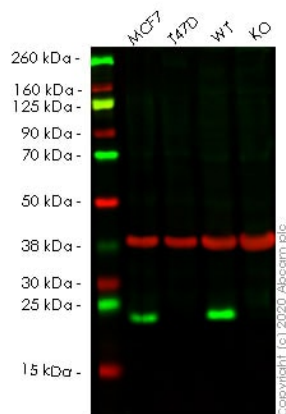
Applications

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab255412 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
Sanger Sequencing		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 122 kDa.



Western blot - Human MUC1 knockout HeLa cell line (ab255412)

All lanes : Anti-MUC1 antibody [EP1024Y] ([ab45167](#)) at 1/1000 dilution

Lane 1 : MCF7 (Human breast adenocarcinoma cell line) cell lysate

Lane 2 : T-47D (Human ductal breast epithelial tumor cell line) cell lysate

Lane 3 : Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate

Lane 4 : MUC1 knockout HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 122 kDa

Observed band size: 24 kDa

Lanes 1- 4: Merged signal (red and green). Green - [ab45167](#) observed at 24 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

[ab45167](#) was shown to react with MUC1 in Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) cells in western blot. Loss of signal was observed when knockout cell line ab255412 (knockout cell lysate [ab263764](#)) was used. Wild-type HeLa (Human epithelial cell line from cervix adenocarcinoma) and MUC1 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. [ab45167](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) overnight at 4°C at a 1 in 1000 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.

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Mut  TCTCCTTTCTTCTGCTGCTGCTCCTCACAAGTGTACAGGTGAGGGGCACGAGGTGGG
      |||
WT   TCTCCTTTCTTCTGCTGCTGCTCCTCACA GTGCTTACAGGTGAGGGGCACGAGGTGGG
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Homozygous: 1 bp insertion in exon 1.

Sanger Sequencing - Human MUC1 knockout HeLa cell line (ab255412)

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