

Product datasheet

Human SIRT5 knockout HeLa cell line ab265617

2 Images

Overview

<b>Product name</b>	Human SIRT5 knockout HeLa cell line
<b>Parental Cell Line</b>	HeLa
<b>Organism</b>	Human
<b>Mutation description</b>	Knockout achieved by using CRISPR/Cas9, 223 bp insertion in exon 5 and Insertion of the selection cassette in exon 5
<b>Passage number</b>	<20
<b>Knockout validation</b>	Sanger Sequencing
<b>Biosafety level</b>	2
<b>General notes</b>	<p><b>Wild-type expression profile:</b> Gene expression value on the wild-type cell line is 0 FPKM (Fragments Per Kilobase Million) for this protein target, according to our RNA-seq data.</p> <p><b>Recommended control:</b> Human wild-type HeLa cell line (<a href="#">ab255928</a>). 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><b>Cryopreservation cell medium:</b> Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose.</p> <p><b>Culture medium:</b> DMEM (High Glucose) + 10% FBS</p> <p><b>Initial handling guidelines:</b> 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"> <li>1. Thaw the vial in 37°C water bath approximately 1-2 minutes.</li> <li>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 <b>culture medium</b>, wash vial with an additional 0.8 ml <b>culture medium</b> (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.</li> <li>3. Resuspend the cell pellet in 5 ml pre-warmed <b>culture medium</b> and count using a haemocytometer (<a href="#">Click here to view haemocytometer protocol</a>) or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of 2x10<sup>4</sup> cells/cm<sup>2</sup>. This should allow for confluency within 48 hours. Seeding density is given as a guide only and should be scaled to align with individual lab schedules.</li> <li>4. Incubate the culture at 37°C incubator with 5% CO<sub>2</sub>. Cultures should be monitored daily.</li> </ol> <p><b>Subculture guidelines:</b></p> <p>All seeding densities should be based on cell counts gained by established methods.</p>

A guide seeding density of  $2 \times 10^4$  cells/cm<sup>2</sup> is recommended for confluency (80-90% confluence) within 48 hours.

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.

[Click here to view the Mammalian cell tissue culture protocol](#)

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## Properties

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<b>Number of cells</b>	1 x 10 <sup>6</sup> cells/vial, 1 mL
<b>Viability</b>	~90%
<b>Adherent /Suspension</b>	Adherent
<b>Tissue</b>	Cervix
<b>Cell type</b>	epithelial
<b>Disease</b>	Adenocarcinoma
<b>Gender</b>	Female
<b>STR Analysis</b>	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
<b>Mycoplasma free</b>	Yes
<b>Storage instructions</b>	Shipped on Dry Ice. Store in liquid nitrogen.
<b>Storage buffer</b>	Constituents: 8.7% DMSO, 2% Cellulose, methyl ether

## Target

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<b>Function</b>	NAD-dependent lysine demalonylase, desuccinylase and deglutarylase that specifically removes malonyl, succinyl and glutaryl groups on target proteins (PubMed:21908771, PubMed:22076378, PubMed:24703693). Activates CPS1 and contributes to the regulation of blood ammonia levels during prolonged fasting: acts by mediating desuccinylation and deglutarylation of CPS1, thereby increasing CPS1 activity in response to elevated NAD levels during fasting (PubMed:22076378, PubMed:24703693). Activates SOD1 by mediating its desuccinylation, leading to reduced reactive oxygen species (PubMed:24140062). Modulates ketogenesis through the desuccinylation and activation of HMGCS2 (By similarity). Has weak NAD-dependent protein deacetylase activity; however this activity may not be physiologically relevant in vivo. Can deacetylate cytochrome c (CYCS) and a number of other proteins in vitro such as UOX.
<b>Tissue specificity</b>	Widely expressed.
<b>Sequence similarities</b>	Belongs to the sirtuin family. Class III subfamily. Contains 1 deacetylase sirtuin-type domain.
<b>Domain</b>	In contrast to class I sirtuins, class III sirtuins have only weak deacetylase activity. Difference in substrate specificity is probably due to a larger hydrophobic pocket with 2 residues (Tyr-102 and Arg-105) that bind to malonylated and succinylated substrates and define the specificity (PubMed:22076378).

## Cellular localization

Mitochondrion; Cytoplasm. Mitochondrion and Mitochondrion matrix. Mitochondrion intermembrane space. Cytoplasm, cytosol. Nucleus. Mainly mitochondrial. Also present extramitochondrially: a fraction is present in the cytosol and very small amounts are also detected in the nucleus.

## Images

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Mut  CGGGCTCACACTCGGCTATGCGCGTCCGGCTCGACATCGGCAAGGTGTGGTCCGGAC
      |||
WT   CGGGCTCACACTCGGCTATG
```

Sanger Sequencing - Human SIRT5 knockout HeLa cell line (ab265617)

Allele-1: 223 bp insertion in exon 5.

```
Mut  GGGTCTCACACTCGGCTATG*****Insertion*****GCGCGTGCCTGGCGTTGGG
      |||
WT   GGGTCTCACACTCGGCTATG          GCGCGTGCCTGGCGTTGGG
```

Sanger Sequencing - Human SIRT5 knockout HeLa cell line (ab265617)

Allele-2: Insertion of the selection cassette in exon 5.

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