

Human SORT1 (Sortilin/NT3) knockout HeLa cell line ab264772

6 Images

Overview

| | |
|-----------------------------|---|
| Product name | Human SORT1 (Sortilin/NT3) knockout HeLa cell line |
| Parental Cell Line | HeLa |
| Organism | Human |
| Mutation description | Knockout achieved by using CRISPR/Cas9, 11 bp deletion in exon 5 and 16 bp deletion in exon 5 and 1 bp insertion in exon 5 and Insertion of the selection cassette in exon 5 |
| Passage number | <20 |
| Knockout validation | Sanger Sequencing, Western Blot (WB) |
| Tested applications | Suitable for: WB |
| Biosafety level | 2 |
| General notes | <p>Recommended control: Human wild-type HeLa cell line (ab255448). 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.</p> |

A guide seeding density of 2×10^4 cells/cm² 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 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

| | |
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| Function | Functions as a sorting receptor in the Golgi compartment and as a clearance receptor on the cell surface. Required for protein transport from the Golgi apparatus to the lysosomes by a pathway that is independent of the mannose-6-phosphate receptor (M6PR). Also required for protein transport from the Golgi apparatus to the endosomes. Promotes neuronal apoptosis by mediating endocytosis of the proapoptotic precursor forms of BDNF (proBDNF) and NGFB (proNGFB). Also acts as a receptor for neurotensin. May promote mineralization of the extracellular matrix during osteogenic differentiation by scavenging extracellular LPL. Probably required in adipocytes for the formation of specialized storage vesicles containing the glucose transporter SLC2A4/GLUT4 (GLUT4 storage vesicles, or GSVs). These vesicles provide a stable pool of SLC2A4 and confer increased responsiveness to insulin. May also mediate transport from the endoplasmic reticulum to the Golgi. |
| Tissue specificity | Expressed at high levels in brain, spinal cord, heart, skeletal muscle, thyroid, placenta and testis. Expressed at lower levels in lymphoid organs, kidney, colon and liver. |
| Involvement in disease | Note=A common polymorphism located in a non-coding region between CELSR2 and PSRC1 alters a CEBP transcription factor binding site and is responsible for changes in hepatic expression of SORT1. Altered SORT1 expression in liver affects low density lipoprotein cholesterol levels in plasma and is associated with susceptibility to myocardial infarction. |
| Sequence similarities | Belongs to the VPS10-related sortilin family. SORT1 subfamily. Contains 9 BNR repeats. |

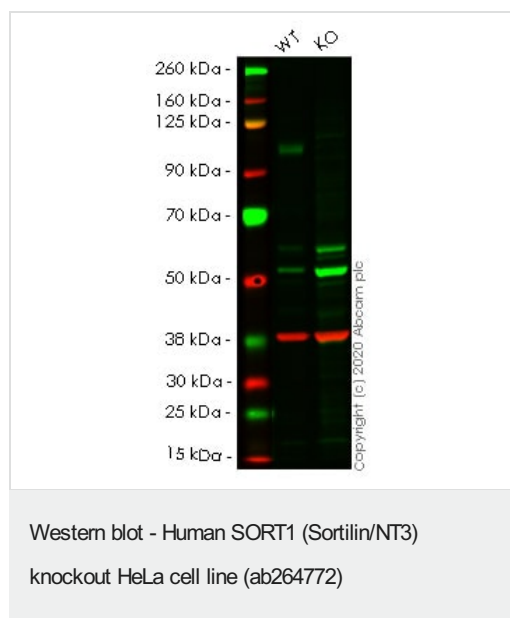
| | |
|---|--|
| Domain | <p>The N-terminal propeptide may facilitate precursor transport within the Golgi stack. Intrachain binding of the N-terminal propeptide and the extracellular domain may also inhibit premature ligand binding.</p> <p>The extracellular domain may be shed following protease cleavage in some cell types.</p> |
| Post-translational modifications | <p>The N-terminal propeptide is cleaved by furin and possibly other homologous proteases.</p> |
| Cellular localization | <p>Membrane. Endoplasmic reticulum membrane. Endosome membrane. Golgi apparatus > Golgi stack membrane. Lysosome membrane. Nucleus membrane. Cell membrane. Lysosome membrane. Localized to membranes of the endoplasmic reticulum, endosomes, Golgi stack, lysosomes and nucleus. A small fraction of the protein is also localized to the plasma membrane. May also be found in SLC2A4/GLUT4 storage vesicles (GSVs) in adipocytes. Localization to the plasma membrane in adipocytes may be enhanced by insulin.</p> |

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab264772 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: 92 kDa. |

Images



All lanes : Anti-Sortilin/NT3 antibody (**ab16640**) at 1 µg/ml

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : SORT1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

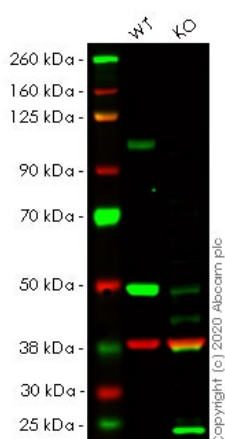
Predicted band size: 92 kDa

Observed band size: 100 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab16640** observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab16640 was shown to react with Sortilin/NT3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout

cell line ab264772 (knockout cell lysate [ab257696](#)) was used. Wild-type HeLa and SORT1 knockout HeLa 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. [ab16640](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated overnight at 4°C at a 1 µg/ml 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.



Western blot - Human SORT1 (Sortilin/NT3)
knockout HeLa cell line (ab264772)

All lanes : Anti-Sortilin/NT3 antibody [EPR15010] ([ab188586](#)) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2 : SORT1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 92 kDa

Observed band size: 100 kDa

Lanes 1- 2: Merged signal (red and green). Green - [ab188586](#) observed at 100 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

[ab188586](#) was shown to react with Sortilin/NT3 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab264772 (knockout cell lysate [ab257696](#)) was used. Wild-type HeLa and SORT1 knockout HeLa 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. [ab188586](#) and Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) were incubated 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.

| | |
|--|--|
| Mut | CTATCAGGTGGTGTAAACAGCA-----GTCGTGGAGGAAGAATCTTCAG |
| | |
| WT | CTATCAGGTGGTGTAAACAGCAGAGGTGTCTGGAGGAAGTCGTGGAGGAAGAATCTTCAG |
| Sanger Sequencing - Human SORT1 knockout HeLa cell line (ab264772) | |

Allele-1: 16 bp deletion in exon 5.

| | |
|--|--|
| Mut | CTATCAGGTGGTGTAAACAGCA-----AGGAAGTCGTGGAGGAAGAATCTTCAG |
| | |
| WT | CTATCAGGTGGTGTAAACAGCAGAGGTGTCTGGAGGAAGTCGTGGAGGAAGAATCTTCAG |
| Sanger Sequencing - Human SORT1 knockout HeLa cell line (ab264772) | |

Allele-2: 11 bp deletion in exon 5.

| | |
|--|--|
| Mut | CTATCAGGTGGTGTAAACAGCAAGAGGTGTCTGGAGGAAGTCGTGGAGGAAGAATCTTCA |
| | |
| WT | CTATCAGGTGGTGTAAACAGCA GAGGTGTCTGGAGGAAGTCGTGGAGGAAGAATCTTCA |
| Sanger Sequencing - Human SORT1 knockout HeLa cell line (ab264772) | |

Allele-3: 1 bp insertion in exon 5.

| | |
|--|---|
| Mut | GTGTTAACAGCAGAGGTGTC*****Insertion*****TGGAGGAAGTCGTGGAGGAA |
| | |
| WT | GTGTTAACAGCAGAGGTGTC TGGAGGAAGTCGTGGAGGAA |
| Sanger Sequencing - Human SORT1 knockout HeLa cell line (ab264772) | |

Allele-4: Insertion of the selection cassette in exon 5.

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