abcam

Product datasheet

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

画像数 6

製品の概要

製品名	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)
アプリケーション	**適用あり:** WB
Biosafety level	2
特記事項	**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. **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.
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.

製品の特性

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
保存方法	Shipped on Dry Ice. Store in liquid nitrogen.
バッファー	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

ターゲット情報

機能	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.
組織特異性	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.
関連疾患	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.
配列類似性	Belongs to the VPS10-related sortilin family. SORT1 subfamily. Contains 9 BNR repeats.
ドメイン	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.

	The extracellular domain may be shed following protease cleavage in some cell types.
翻訳後修飾	The N-terminal propeptide is cleaved by furin and possibly other homologous proteases.
細胞内局在	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.

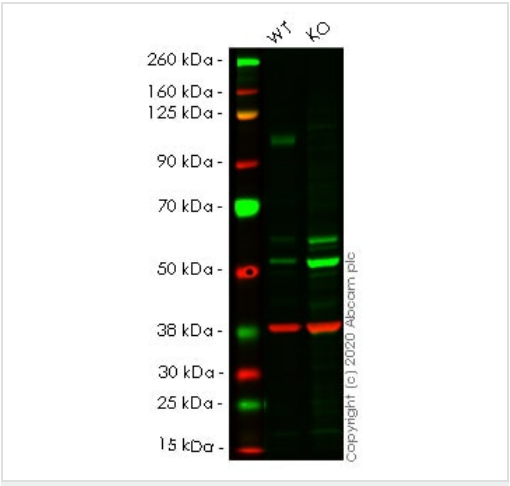
アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab264772の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 92 kDa.

画像



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

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.

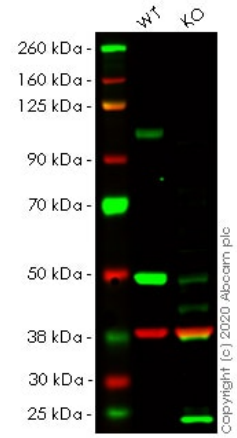
Performed under reducing conditions.

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

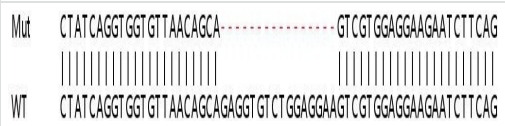
Lysates/proteins at 20 µg per lane.

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.



Sanger Sequencing - Human SORT1 knockout HeLa cell line (ab264772)

Allele-1: 16 bp deletion in exon 5.

Sanger Sequencing - Human SORT1 knockout HeLa cell line (ab264772)

Allele-2: 11 bp deletion in exon 5.

Sanger Sequencing - Human SORT1 knockout HeLa cell line (ab264772)

Allele-3: 1 bp insertion in exon 5.

Sanger Sequencing - Human SORT1 knockout HeLa cell line (ab264772)

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

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