abcam

Product datasheet

Human SCD (SCD1) knockout HeLa cell line ab265220

1 References　画像数 7

製品の概要

製品名	Human SCD (SCD1) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 4 bp deletion in exon 3 and Insertion of the selection cassette in exon 3
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 (**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.
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	Terminal component of the liver microsomal stearyl-CoA desaturase system, that utilizes O(2) and electrons from reduced cytochrome b5 to catalyze the insertion of a double bond into a spectrum of fatty acyl-CoA substrates including palmitoyl-CoA and stearoyl-CoA.
配列類似性	Belongs to the fatty acid desaturase family.
ドメイン	The histidine box domains may contain the active site and/or be involved in metal ion binding.
細胞内局在	Endoplasmic reticulum membrane.

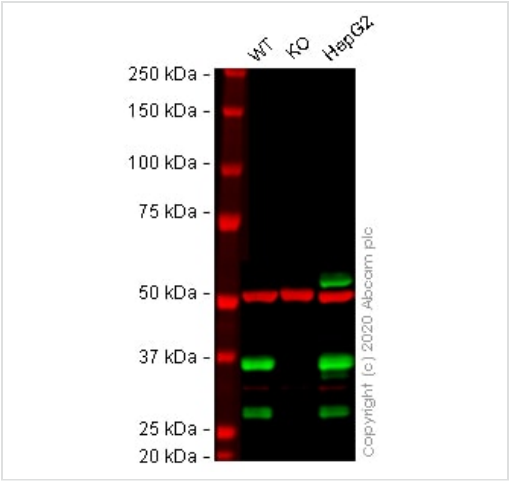
アプリケーション

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ab265220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: 42 kDa.

画像



Western blot - Human SCD (SCD1) knockout HeLa cell line (ab265220)

All lanes : Anti-SCD1 antibody [CD.E10] (**ab19862**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : SCD knockout HeLa cell lysate
Lane 3 : HepG2 cell lysate

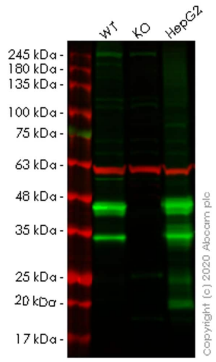
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 42 kDa
Observed band size: 36 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab19862** observed at 36 kDa. Red - loading control **ab2866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) observed at 55kDa.

ab19862 was shown to react with SCD1 in HeLa wild-type cells in western blot with loss of signal observed in SCD knockout cell line ab265220 (SCD knockout cell lysate **ab257658**). HeLa wild-type and SCD knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with **ab19862** and **ab2866** (Rabbit anti-alpha Tubulin antibody [EP1332Y]) overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye® 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Human SCD knockout HeLa cell line (ab265220)

All lanes : Anti-SCD1 antibody [EPR21963] (**ab236868**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : SCD knockout HeLa cell lysate
Lane 3 : HepG2 cell lysate

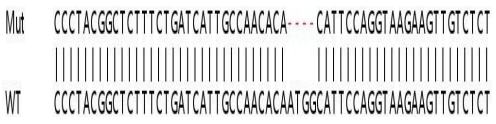
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 42 kDa

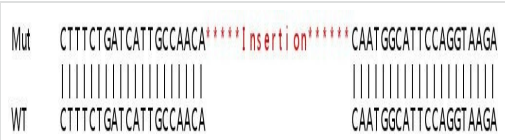
anes 1-3: Merged signal (red and green). Green - **ab236868**. Red - loading control **ab8245** observed at 50 kDa.

ab236868 Anti-SCD1 antibody [EPR21963] was shown to specifically react with SCD1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265220 (knockout cell lysate **ab257658**) was used. Wild-type and SCD1 knockout samples were subjected to SDS-PAGE. **ab236868** and Anti-tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at 1 in 1000 dilution and 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 10000 dilution for 1 hour at room temperature before imaging.



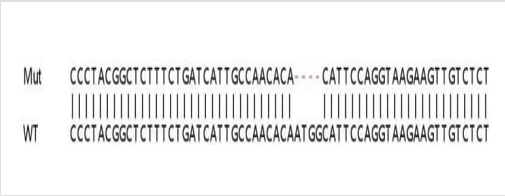
Sanger Sequencing - Human SCD knockout HeLa cell line (ab265220)

Allele-1: 4 bp deletion in exon 3.



Sanger Sequencing - Human SCD knockout HeLa cell line (ab265220)

Allele-2: 4 bp deletion in exon 3.



Sanger Sequencing - Human SCD knockout HeLa cell line (ab265220)

Allele-3: 4 bp deletion in exon 3.

Sanger Sequencing - Human SCD knockout HeLa cell line (ab265220)

Allele-5: Insertion of the selection cassette in exon 3.

Sanger Sequencing - Human SCD knockout HeLa cell line (ab265220)

Allele-4: Insertion of the selection cassette in exon 3.

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