abcam

Product datasheet

Human SIPA1 (Spa-1) knockout HeLa cell line ab265434

4 Images

Overview

Product name	Human SIPA1 (Spa-1) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 182 bp deletion in exon 5 and 1 bp deletion in exon 5 and 4 bp deletion in exon 5
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	2
General notes	**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 and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

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 vWA: 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	GTPase activator for the nuclear Ras-related regulatory proteins Rap1 and Rap2 in vitro, converting it to the putatively inactive GDP-bound state.
Tissue specificity	Expressed in fetal as well as in adult tissues. Expressed abundantly in the lymphoid tissues such as thymus, spleen and peripheral blood lymphocytes and also shows a significant expression in the spinal cord.
Sequence similarities	Contains 1 PDZ (DHR) domain. Contains 1 Rap-GAP domain.
Cellular localization	Nucleus. Cytoplasm > perinuclear region. Endomembrane system. Mostly localized in the perinuclear membraneous region.

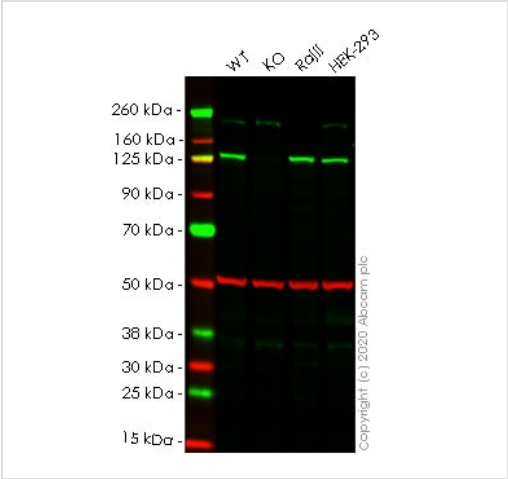
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab265434 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: 112 kDa.

Images



Western blot - Human SIPA1 (Spa-1) knockout HeLa cell line (ab265434)

All lanes : Anti-Spa-1 antibody [EPR14134] (**ab189929**) at 1/5000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : Spa-1 knockout HeLa cell lysate
Lane 3 : Raji cell lysate
Lane 4 : HEK-293 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 112 kDa
Observed band size: 130 kDa

Lanes 1-4: Merged signal (red and green). Green - **ab189929** observed at 130 kDa. Red - loading control **ab7291** observed at 50 kDa.

ab189929 Anti-Spa-1 antibody [EPR14134] was shown to specifically react with Spa-1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265434 (knockout cell lysate **ab258189**) was used. Wild-type and Spa-1 knockout samples were subjected to SDS-PAGE. **ab189929** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at 1 in 5000 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 20000 dilution for 1 hour at room temperature before imaging.

Sanger Sequencing - Human SIPA1 knockout HeLa cell line (ab265434)

Allele-1: 4 bp deletion in exon 5.

Sanger Sequencing - Human SIPA1 knockout HeLa cell line (ab265434)

Allele-2: 1 bp deletion in exon 5.

Sanger Sequencing - Human SIPA1 knockout HeLa cell line (ab265434)

Allele-3: 182 bp deletion in exon 5.

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