abcam

Product datasheet

Human CAV1 (Caveolin-1) knockout HeLa cell line ab255371

8 Images

Overview

Product name	Human CAV1 (Caveolin-1) knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, 1 bp insertion in exon 1 and Insertion of the selection cassette in exon 1
Passage number	<20
Knockout validation	Immunocytochemistry (ICC), Sanger Sequencing, Western Blot (WB)
Tested applications	**Suitable for:** Flow Cyt, ICC, WB
Biosafety level	2
General notes	**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.

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	May act as a scaffolding protein within caveolar membranes. Interacts directly with G-protein alpha subunits and can functionally regulate their activity (By similarity). Involved in the costimulatory signal essential for T-cell receptor (TCR)-mediated T-cell activation. Its binding to DPP4 induces T-cell proliferation and NF-kappa-B activation in a T-cell receptor/CD3-dependent manner. Recruits CTNNB1 to caveolar membranes and may regulate CTNNB1-mediated signaling through the Wnt pathway.
Tissue specificity	Expressed in muscle and lung, less so in liver, brain and kidney.
Involvement in disease	Defects in CAV1 are the cause of congenital generalized lipodystrophy type 3 (CGL3) [MIM:612526]; also called Berardinelli-Seip congenital lipodystrophy type 3 (BSCL3). Congenital generalized lipodystrophies are autosomal recessive disorders characterized by a near absence of adipose tissue, extreme insulin resistance, hypertriglyceridemia, hepatic steatosis and early onset of diabetes.
Sequence similarities	Belongs to the caveolin family.
Post-translational modifications	The initiator methionine for isoform Beta is removed during or just after translation. The new N-terminal amino acid is then N-acetylated.
Cellular localization	Golgi apparatus membrane. Cell membrane. Membrane > caveola. Membrane raft. Colocalized with DPP4 in membrane rafts. Potential hairpin-like structure in the membrane. Membrane protein of caveolae.

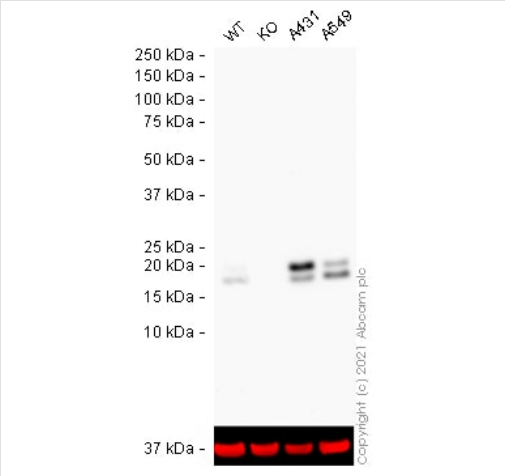
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab255371 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
Flow Cyt		Use at an assay dependent concentration.
ICC		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 20 kDa.

Images



Western blot - Human CAV1 (Caveolin-1) knockout HeLa cell line (ab255371)

All lanes : HRP Anti-Caveolin-1 antibody [E249] - Caveolae Marker (**ab193893**) at 1/5000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CAV1 knockout HeLa cell lysate
Lane 3 : A431 cell lysate
Lane 4 : A549 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

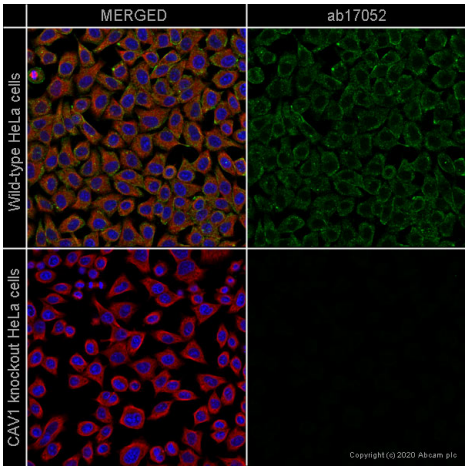
Predicted band size: 20 kDa
Observed band size: 20 kDa

Exposure time: 90 seconds

Lanes 1 - 4: Merged signal (red and green). Green - **ab193893** observed at 20 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

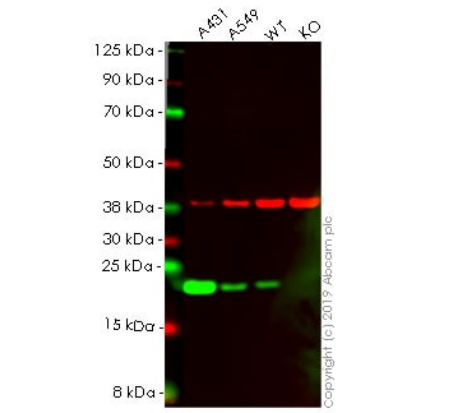
ab193893 was shown to react with Caveolin-1 in wild-type HeLa cells in Western blot with loss of signal observed in CAV1 knockout cell line ab255371 (CAV1 knockout cell lysate **ab263806**). Wild-type HeLa and CAV1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with **ab193893** and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 5000

dilution and a 1 in 20000 dilution respectively. Blots were incubated with and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature. Blots were developed with Optiblot ECL reagent (**ab133456**) before imaging.



Immunocytochemistry/ Immunofluorescence - Human CAV1 (Caveolin-1) knockout HeLa cell line (ab255371)

ab17052 staining Caveolin-1 in wild-type HeLa cells (top panel) and CAV1 knockout HeLa cells (ab255371) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab17052** at 1/500 dilution and **ab6046** (Rabbit polyclonal to beta Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to mouse IgG (Alexa Fluor® 488) (**ab150117**) at 2 µg/ml (shown in green) and a goat secondary antibody to rabbit IgG (Alexa Fluor® 594) (**ab150080**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).



Western blot - Human CAV1 (Caveolin-1) knockout HeLa cell line (ab255371)

All lanes : Anti-Caveolin-1 antibody [EPR15554] - N-terminal (**ab192869**) at 1/10000 dilution

Lane 1 : A431 cell lysate
Lane 2 : A549 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : CAV1 knockout HeLa 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

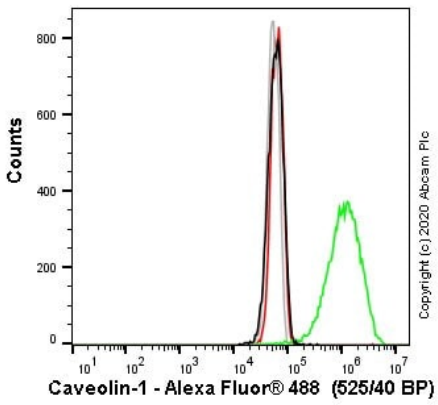
Performed under reducing conditions.

Predicted band size: 20 kDa
Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - **ab192869**

observed at 20 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab192869 was shown to react with Caveolin-1 in wild-type HeLa. Loss of signal was observed when knockout cell line ab255371 (knockout cell lysate **ab263806**) was used. Wild-type and Caveolin-1 knockout samples were subjected to SDS-PAGE. **ab192869** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 10000 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.



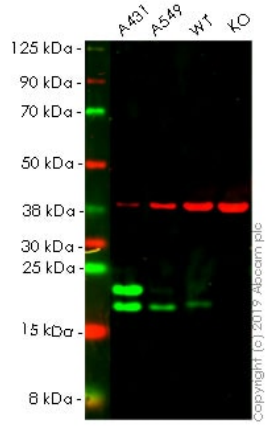
Flow Cytometry - Human CAV1 (Caveolin-1) knockout HeLa cell line (ab255371)

Flow cytometry overlay histogram showing wild-type HeLa (green line) and CAV1 knockout HeLa cells (ab255371) stained with **ab192869** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab192869**) ($1x10^6$ in 100µl at 0.04 µg/ml) for 30 min at 22°C.

The secondary antibody Goat anti-rabbit IgG H&L (Alexa Fluor® 488, pre-adsorbed) (**ab150081**) was used at 1/2000 for 30 min at 22°C.

Isotype control antibody was Rabbit IgG (monoclonal) (**ab172730**) used at the same concentration and conditions as the primary antibody (wild-type HeLa - black line CAV1 knockout HeLa - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Human CAV1 (Caveolin-1) knockout HeLa cell line (ab255371)

All lanes : Anti-Caveolin-1 antibody [E249] - Caveolae Marker (**ab32577**) at 1/1000 dilution

Lane 1 : A431 cell lysate
Lane 2 : A549 cell lysate
Lane 3 : Wild-type HeLa cell lysate
Lane 4 : CAV1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

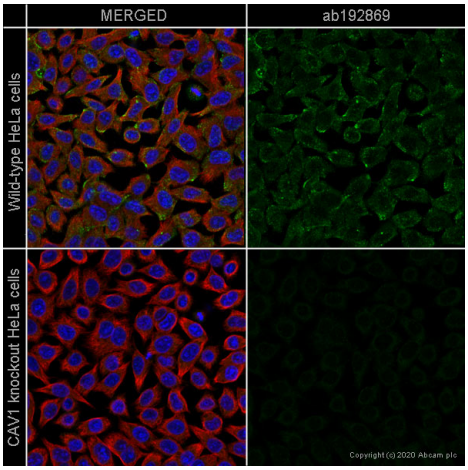
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 20 kDa
Additional bands at: 37 kDa (possible Loading Control)

Lanes 1 - 4: Merged signal (red and green). Green - **ab32577** observed at 20 kDa. Red - loading control, **ab8245** observed at 37 kDa.

ab32577 was shown to react with Caveolin-1 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab255371 (knockout cell lysate **ab263806**) was used. Wild-type and Caveolin-1 knockout samples were subjected to SDS-PAGE. **ab32577** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) 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 20000 dilution for 1 hour at room temperature before imaging.

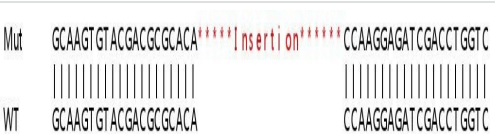


Immunocytochemistry/ Immunofluorescence - Human CAV1 (Caveolin-1) knockout HeLa cell line (ab255371)

ab192869 staining Caveolin-1 in wild-type HeLa cells (top panel) and CAV1 knockout HeLa cells (ab255371) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab192869** at 1/500 dilution and **ab7291** (Mouse monoclonal to alpha Tubulin) at 1/1000 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat secondary antibody to rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green) and a goat secondary antibody to mouse IgG (Alexa Fluor® 594) (**ab150120**) at 2 µg/ml (shown in red). Nuclear DNA was labelled in blue with DAPI.
Image was taken with a confocal microscope (Leica-Microsystems TCS SP8).

Sanger Sequencing - Human CAV1 knockout HeLa cell line (ab255371)

Allele-1: 1 bp insertion in exon 1.



Sanger Sequencing - Human CAV1 knockout HeLa cell line (ab255371)

Allele-2: Insertion of the selection cassette in exon 1.

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