abcam

Product datasheet

Anti-Caveolin-1 antibody [EPR15554] - N-terminal ab192869

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-Caveolin-1 antibody [EPR15554] - N-terminal
Description	Rabbit monoclonal [EPR15554] to Caveolin-1 - N-terminal
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, Flow Cyt (Intra), WB, IHC-P, IP
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: PC-3, A549, A431 and HeLa cell lysates. IHC-P: Human liver and squamous cell carcinoma of cervix tissues; mouse lung tissue. ICC/IF: A673 and HeLa cells. Flow Cyt (intra): NIH3T3 and HeLa cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR15554
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab192869 in the following tested applications.

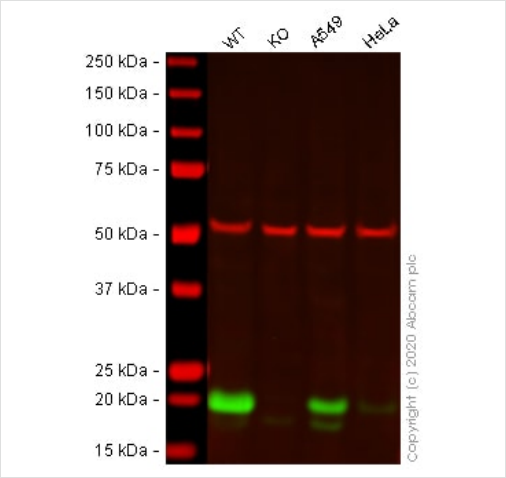
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF		1/300.
Flow Cyt (Intra)		1/120. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB		1/10000 - 1/50000. Detects a band of approximately 17, 20 kDa (predicted molecular weight: 17, 20 kDa).
IHC-P	★★★★★ (2)	1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IP		1/30.

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.

Images



Western blot - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

All lanes : Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869) at 1/1000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : CAV1 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

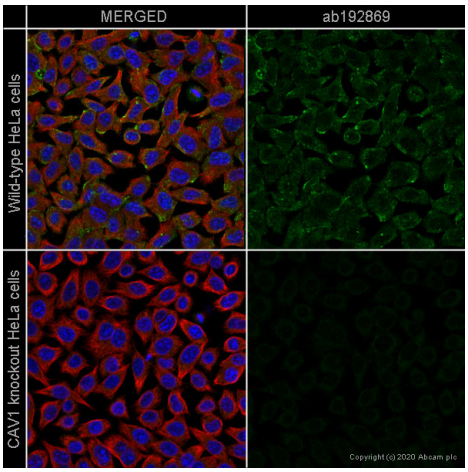
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 17, 20 kDa
Observed band size: 21-24 kDa

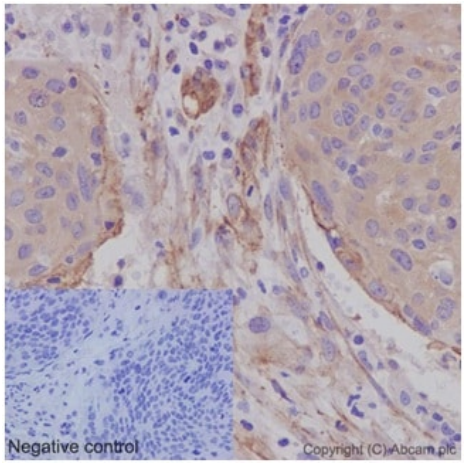
Lanes 1 - 4: Merged signal (red and green). Green - ab192869 observed at 21-24 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab192869 was shown to react with Caveolin-1 in wild-type A431 cells in western blot. Loss of signal was observed when CAV1 knockout sample was used. Wild-type and CAV1 knockout A431 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBS-T (0.1% Tween®) before incubation with ab192869 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 1000 Dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

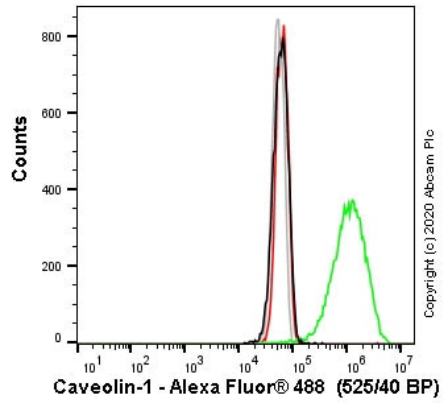
ab192869 staining Caveolin-1 in wild-type HeLa cells (top panel) and CAV1 knockout HeLa cells (**ab255371**) (bottom panel). The cells were fixed with 100% methanol (5 min) then permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% 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).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

Immunohistochemical analysis of paraffin-embedded
Human squamous cell carcinoma of cervix tissue labeling Caveolin-1 using ab192869 at 1/4000 dilution (0.15 µg/ml). A prediluted HRP Polymer for Rabbit IgG was used as the secondary. Counterstain: Hematoxylin. Negative control also shown.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



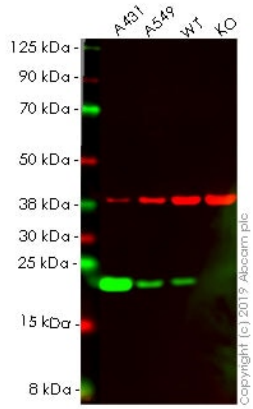
Flow Cytometry (Intracellular) - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

Intracellular Intracellular 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 Fluorr® 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 - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

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.

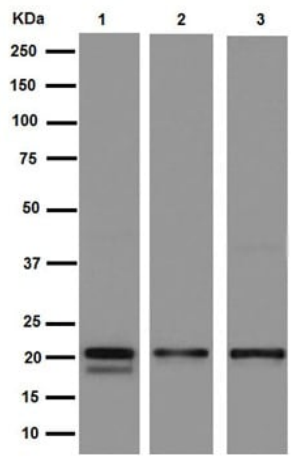
Performed under reducing conditions.

Predicted band size: 17, 20 kDa

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.



Western blot - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

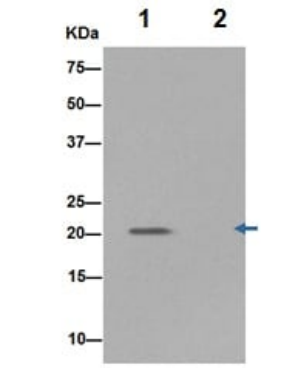
All lanes : Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869) at 1/10000 dilution

Lane 1 : PC-3 cell lysate
Lane 2 : A549 cell lysate
Lane 3 : A431 cell lysate

Lysates/proteins at 10 µg per lane.

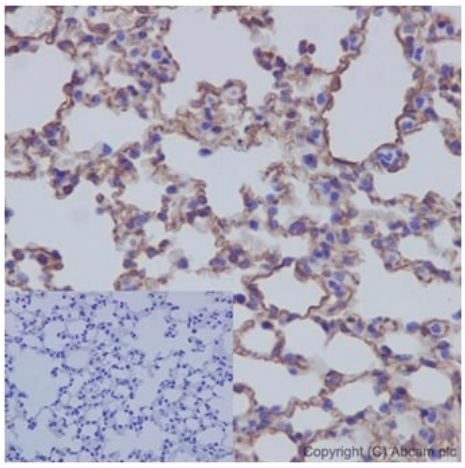
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 17, 20 kDa



Immunoprecipitation - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

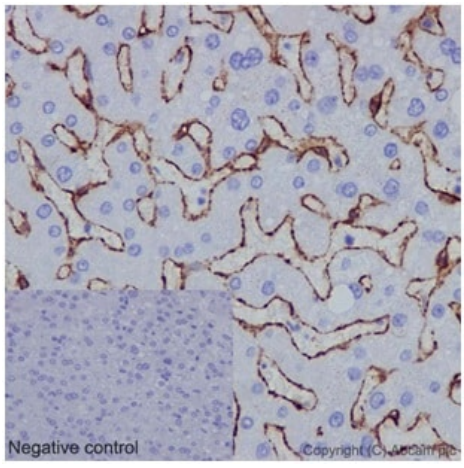
Immunoprecipitation analysis of A431 cell lysate labeling Caveolin-1 using ab192869 at 1/30 dilution (Lane 1). PBS negative control (Lane 2). Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/1500 was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

Immunohistochemical analysis of paraffin-embeddedMouse lung tissue labeling Caveolin-1 using ab192869 at 1/4000 dilution (0.15 µg/ml). A prediluted HRP Polymer for Rabbit IgG was used as the secondary. Counterstain: Hematoxylin. Negative control also shown.

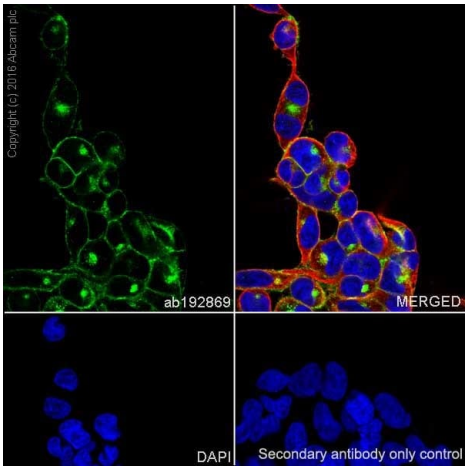
Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

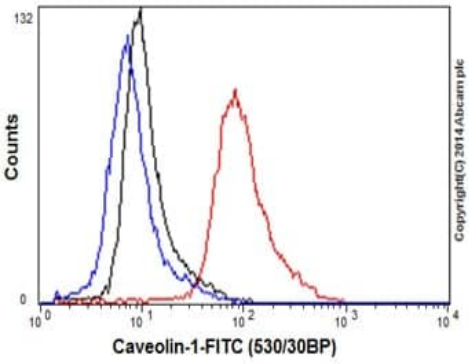
Immunohistochemical analysis of paraffin-embedded Human liver tissue labeling Caveolin-1 using ab192869 at 1/4000 dilution (0.15 µg/ml). A prediluted HRP Polymer for Rabbit IgG was used as the secondary. Counterstain: Hematoxylin. Negative control also shown.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

Immunocytochemistry/Immunofluorescence analysis of A-673 cells labelling Caveolin-1 with ab192869 at a dilution of 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000) was used as the secondary antibody. The cells were co-stained with **ab195889**, Alexa Fluor® 594-conjugated anti-Tubulin [DM1A] at a dilution of 1/200. Nuclei counterstained with DAPI (blue).



Flow Cytometry (Intracellular) - Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

Intracellular Flow Cytometry analysis of NIH3T3 cells labeling Caveolin-1 using ab192869 at a 1/120 dilution (Red). A Goat anti rabbit IgG (FITC) at 1/150 dilution was used as secondary antibody. Cells were fixed with 2% paraformaldehyde. Cells without incubation with primary antibody and secondary antibody Blue. Rabbit monoclonal IgG was used as isotype control (Black).

Anti-Caveolin-1 antibody [EPR15554] - N-terminal (ab192869)

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