abcam

Product datasheet

Anti-Caveolin-1 antibody [E249] - Caveolae Marker ab32577

KO VALIDATED Recombinant RabMAb

★★★★★ 2 Abreviews 19 References 11 Images

Overview

Product name	Anti-Caveolin-1 antibody [E249] - Caveolae Marker
Description	Rabbit monoclonal [E249] to Caveolin-1 - Caveolae Marker
Host species	Rabbit
Specificity	This antibody should recognize both alpha and beta form of Caveolin-1.
Tested applications	**Suitable for:** IHC-P, ICC/IF, Flow Cyt, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A431, A549 and HeLa cell lysates. ICC/IF: HeLa and Jurkat 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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E249

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab32577 in the following tested applications.

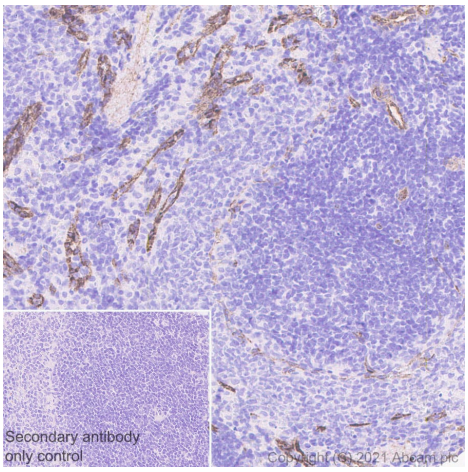
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P	★★★★★ (1)	1/2000. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Use 0.01M Sodium Citrate Buffer, pH 6.0. For unpurified use at 1:250.
ICC/IF		1/50.
Flow Cyt		1/20.
WB	★★★★★ (1)	1/1000 - 1/10000. Predicted molecular weight: 20 kDa.

Target

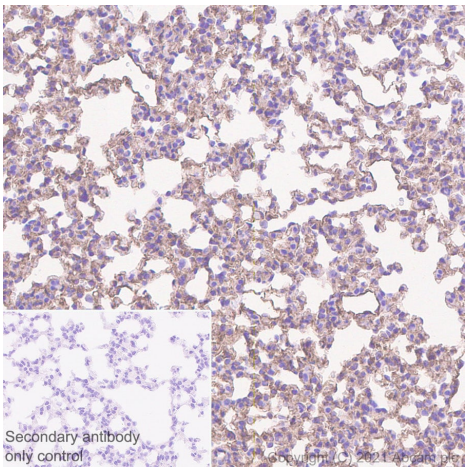
Function	May act as a scaffolding protein within caveolar membranes. Interacts directly with G-protein alpha subunits and can functionally regulate their activity (By similarity). Involved in the costimulatory signal essential for T-cell receptor (TCR)-mediated T-cell activation. Its binding to DPP4 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



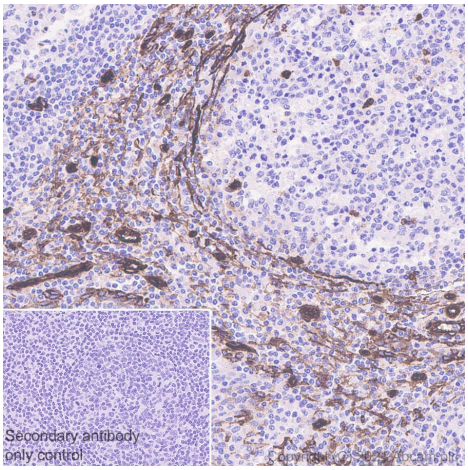
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat spleen tissue sections labelling Caveolin-1 with purified ab32577 at 1/2000 dilution (0.06 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



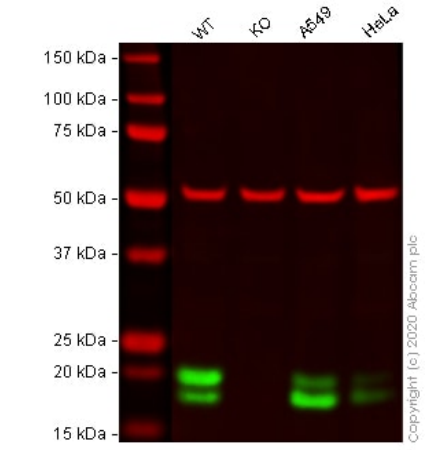
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse lung tissue sections labelling Caveolin-1 with purified ab32577 at 1/2000 dilution (0.06 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human tonsil tissue sections labelling Caveolin-1 with purified ab32577 at 1/2000 dilution (0.06 µg/ml). Heat mediated antigen retrieval was performed using Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0) . Tissue was counterstained with Hematoxylin. Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) secondary antibody was used at 1:0 dilution. PBS instead of the primary antibody was used as the negative control. The immunostaining was performed on a Leica Biosystems BOND® RX instrument.



Western blot - Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

All lanes : Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577) 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

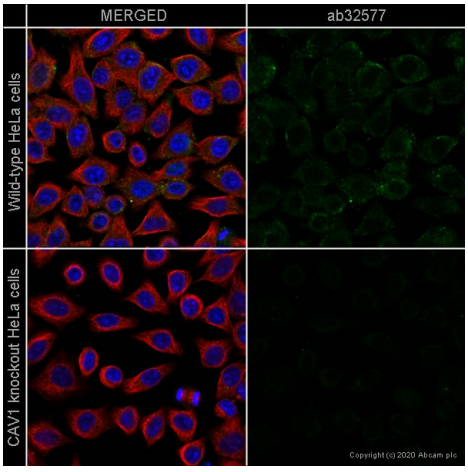
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

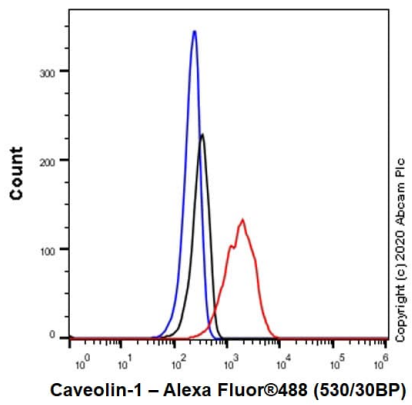
Lanes 1 - 4: Merged signal (red and green). Green - ab32577 observed at 20 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab32577 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. A431 wild-type 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 ab32577 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.



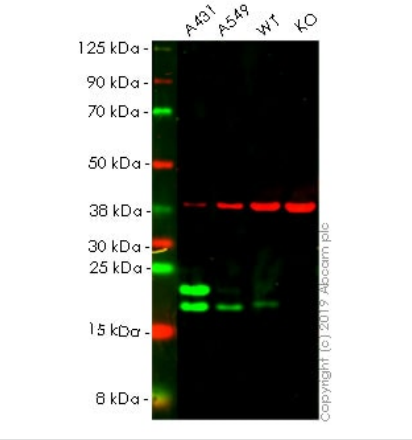
Immunocytochemistry/ Immunofluorescence - Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

ab32577 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 ab32577 at 1/200 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).



Flow Cytometry - Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

Flow cytometric analysis of 4% Paraformaldehyde fixed 90% Methanol permeabilized A431 (Human epidermoid carcinoma epithelial cell) cells labelling Caveolin-1 with ab32577 at 1/20 dilution (10 µg/ml) compared with a Rabbit monoclonal IgG (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 was used as the secondary antibody.



Western blot - Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

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.

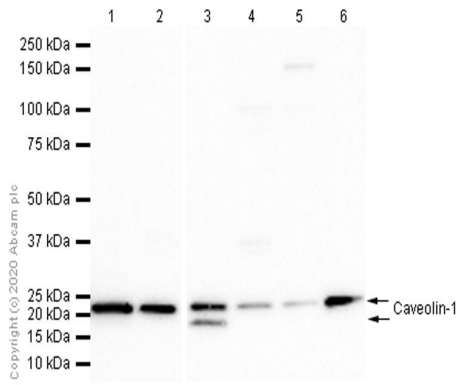
Performed under reducing conditions.

Predicted band size: 20 kDa

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.



Western blot - Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

All lanes :

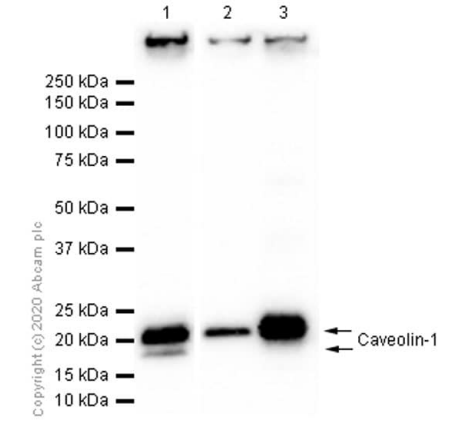
Lane 1 : Mouse heart lysate
Lane 2 : Mouse skeletal muscle lysate
Lane 3 : Rat heart lysate
Lane 4 : Rat skeletal muscle lysate
Lane 5 : C2C12 (Mouse myoblasts myoblast) whole cell lysate
Lane 6 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 20 kDa
Observed band size: 17, 23 kDa

The two isoforms of Caveolin-1 have been described in the literatures (PMID: 12816877, 11748292 and 14992406). We are unsure about the nature of the 250kDa bands.



Western blot - Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

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

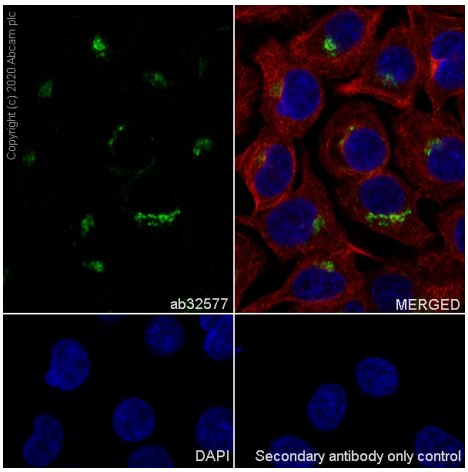
Lane 1 : A431 (Human epidermoid carcinoma epithelial cell) whole cell lysate
Lane 2 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 3 : HaCaT (Human skin keratinocyte) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

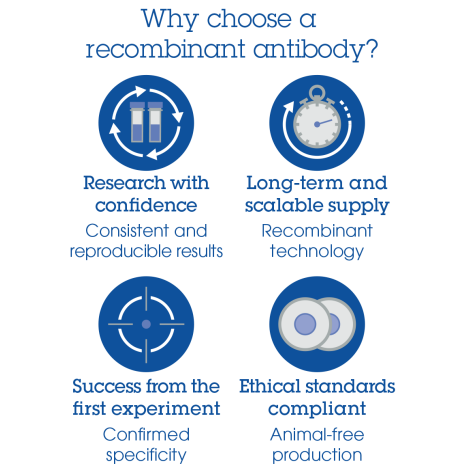
Predicted band size: 20 kDa
Observed band size: 17, 23 kDa

The two isoforms of Caveolin-1 have been described in the literatures (PMID: 12816877, 11748292 and 14992406). We are unsure about the nature of the 250kDa bands.



Immunocytochemistry analysis of Jurkat (Human lung carcinoma epithelial cell) cells labeling Caveolin-1 with purified ab32577 at 1/50 dilution (2.3 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150078**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Immunocytochemistry/ Immunofluorescence - Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)



Anti-Caveolin-1 antibody [E249] - Caveolae Marker (ab32577)

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