abcam

Product datasheet

Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free ab240332

KO VALIDATED Recombinant RabMAb

11 Images

Overview

Product name	Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free
Description	Rabbit monoclonal [EPR15554] to Caveolin-1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), IP, IHC-P, ICC, WB
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A549, A431 and HeLa cell lysates. ICC: HeLa and A763 cells. IHC-P: Human liver and squamous cell carcinoma of cervix tissue; Mouse lung tissue. Flow Cyt (intra): NIH3T3 and HeLa cells.
General notes	ab240332 is the carrier-free version of ab192869. Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR15554
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab240332 in the following tested applications.

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

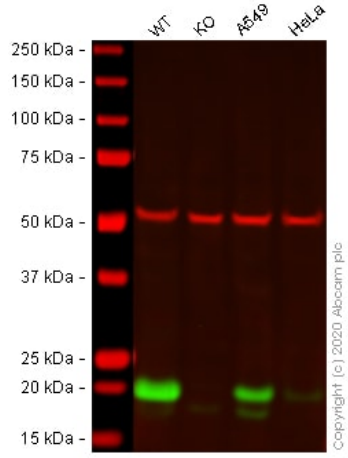
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration. ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 17, 20 kDa (predicted molecular weight: 17, 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



Western blot - Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free (ab240332)

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

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

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

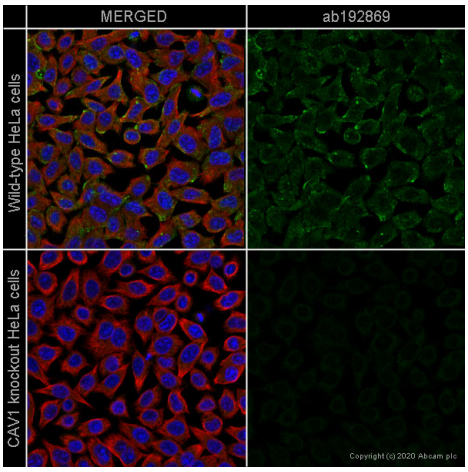
Predicted band size: 17, 20 kDa
Observed band size: 21-24 kDa

This data was developed using the same antibody clone in a different buffer formulation (ab192869).

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 A431 wild-type 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 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.

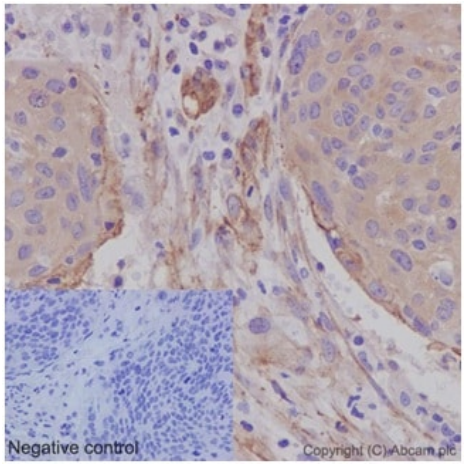


Immunocytochemistry - Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free (ab240332)

This data was developed using the same antibody clone in a different buffer formulation (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).

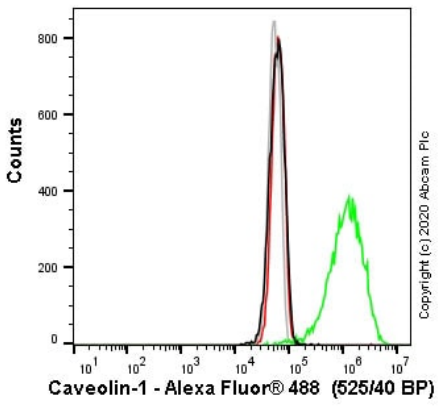


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free (ab240332)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab192869).

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



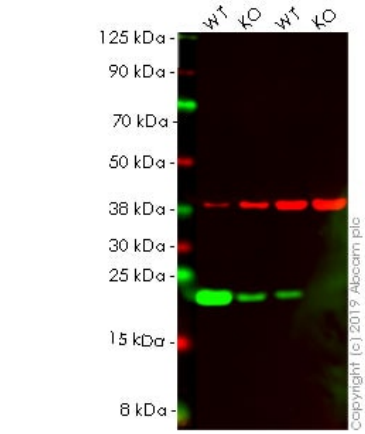
Flow Cytometry (Intracellular) - Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free (ab240332)

Intracellular Intracellular Flow Cytometry overlay histogram showing wild-type HeLa (green line) and CAV1 knockout HeLa cells (ab255371) stained with ab240332 (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 (ab240332) ($1x10^6$ 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 - Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free (ab240332)

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 : Caveolin-1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

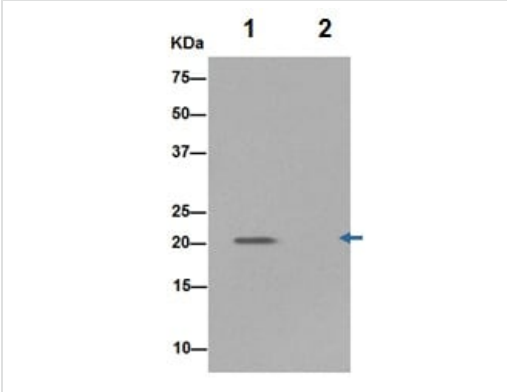
Predicted band size: 17, 20 kDa
Observed band size: 20 kDa

This data was developed using the same antibody clone in a

different buffer formulation (ab192869).

Lanes 1 - 4: Merged signal (red and green). Green - ab192869 observed at 20 kDa. Red - loading control, ab8245 observed at 37 kDa.

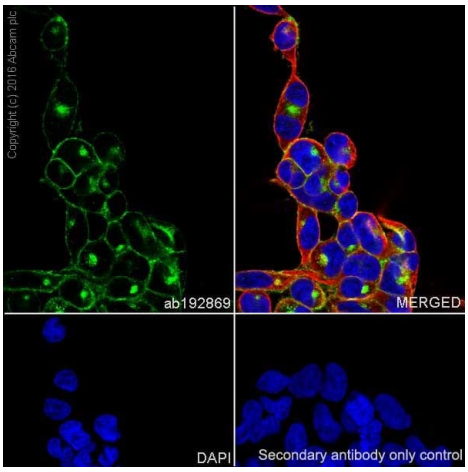
ab192869 was shown to react with Caveolin-1 in wild-type HeLa. Loss of signal was observed when knockout cell line ab255371 (knockout cell lysate ab263806) was used. Wild-type and Caveolin-1 knockout samples were subjected to SDS-PAGE. ab192869 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245)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



Immunoprecipitation - Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free (ab240332)

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.

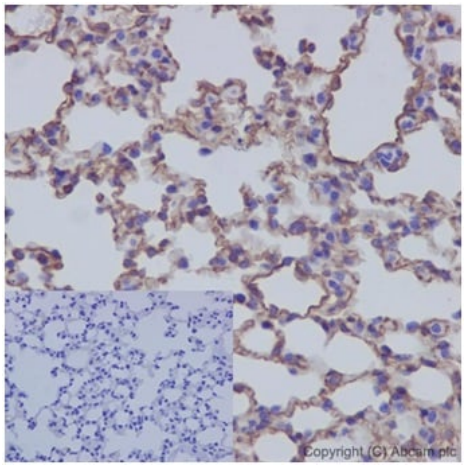
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab192869).



Immunocytochemistry - Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free (ab240332)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab192869).

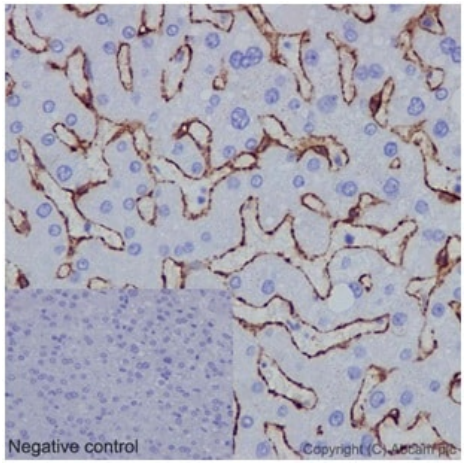


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free (ab240332)

Immunohistochemical analysis of paraffin-embedded Mouse 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab192869).

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

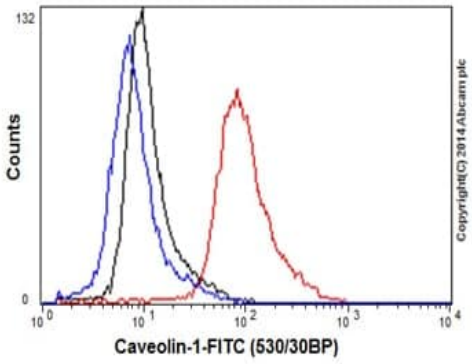


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free (ab240332)

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab192869).

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] - BSA and Azide free (ab240332)

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).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab192869).

Anti-Caveolin-1 antibody [EPR15554] - BSA and Azide free (ab240332)

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