abcam

Product datasheet

Anti-Syntenin antibody [EPR8102] ab133267

KO VALIDATED Recombinant RabMAb

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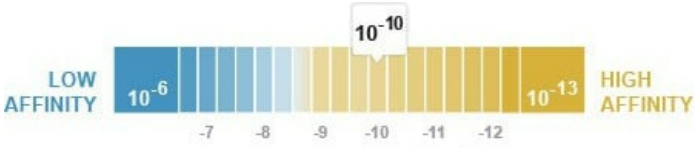
Overview

Product name	Anti-Syntenin antibody [EPR8102]
Description	Rabbit monoclonal [EPR8102] to Syntenin
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide within Human Syntenin aa 1-100. The exact sequence is proprietary. Database link: O00560
Positive control	Human fetal brain lysate, Human fetal heart lysate, Human placenta lysate, HeLa, 293T and A549 (Human lung carcinoma cell line) cell lysates; Human brain tissue
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. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.** Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

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. Avoid freeze / thaw cycle.

Dissociation constant (K_D)	$K_D = 1.24 \times 10^{-10}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR8102
Isotype	IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab133267 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)		1/50. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody. For unpurified use at 1/1000 - 1/10000.
WB	★★★☆☆ (1)	1/1000 - 1/10000. Detects a band of approximately 32 kDa (predicted molecular weight: 32 kDa).
IP		1/10 - 1/100.
IHC-P		1/50 - 1/100. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF		1/50 - 1/200.

Target

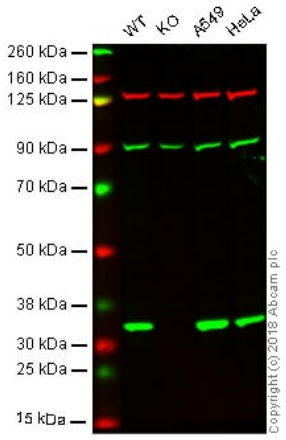
Function	Seems to function as an adapter protein. In adherens junctions may function to couple syndecans to cytoskeletal proteins or signaling components. Seems to couple transcription factor SOX4 to the IL-5 receptor (IL5RA). May also play a role in vesicular trafficking. Seems to be required for the targeting of TGFA to the cell surface in the early secretory pathway.
Tissue specificity	Widely expressed. Expressed in fetal kidney, liver, lung and brain. In adult highest expression in heart and placenta.
Sequence similarities	Contains 2 PDZ (DHR) domains.
Post-translational	Phosphorylated on tyrosine residues.

modifications

Cellular localization

Cell junction > focal adhesion. Cell junction > adherens junction. Cell membrane. Endoplasmic reticulum membrane. Nucleus. Melanosome. Cytoplasm > cytosol. Cytoplasm > cytoskeleton. Mainly membrane-associated. Localized to adherens junctions, focal adhesions and endoplasmic reticulum. Colocalized with actin stress fibers. Also found in the nucleus. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

Images

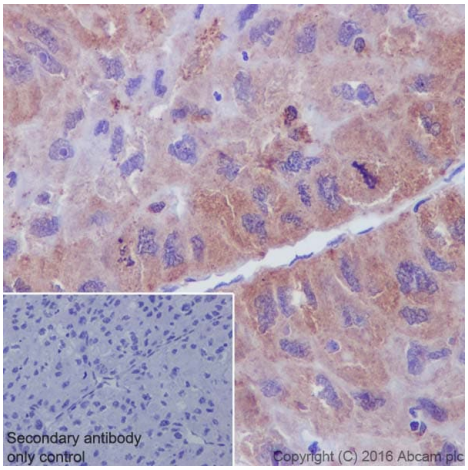


Western blot - Anti-Syntenin antibody [EPR8102] (ab133267)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)
Lane 2: Syntenin knockout HAP1 whole cell lysate (20 µg)
Lane 3: A549 whole cell lysate (20 µg)
Lane 4: HeLa whole cell lysate (20 µg)

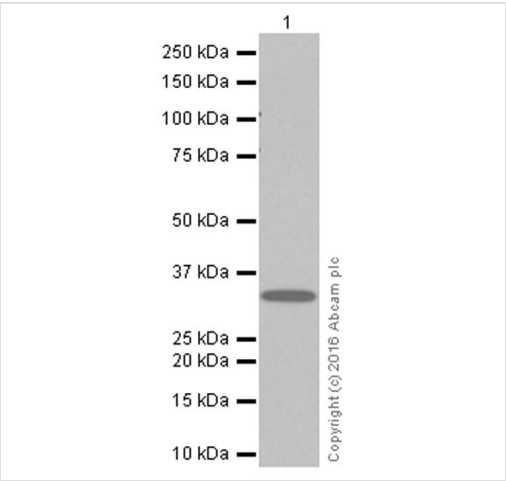
Lanes 1 - 4: Merged signal (red and green). Green - ab133267 observed at 32 kDa. Red - loading control, ab130007, observed at 130 kDa.

ab133267 was shown to recognize Syntenin in wild-type HAP1 cells as signal was lost at the expected MW in Syntenin knockout cells. Additional cross-reactive bands were observed in the wild-type and knockout cells. Wild-type and Syntenin knockout samples were subjected to SDS-PAGE. ab133267 and ab130007 (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed 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 1/20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Syntenin antibody [EPR8102] (ab133267)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human hepatocellular carcinoma tissue labeling Syntenin with purified ab133267 at 1/50 dilution. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin



Western blot - Anti-Syntenin antibody [EPR8102] (ab133267)

Anti-Syntenin antibody [EPR8102] (ab133267) at 1/2000 dilution (purified) + A549 (Human lung carcinoma cell line) whole cell lysate at 15 µg

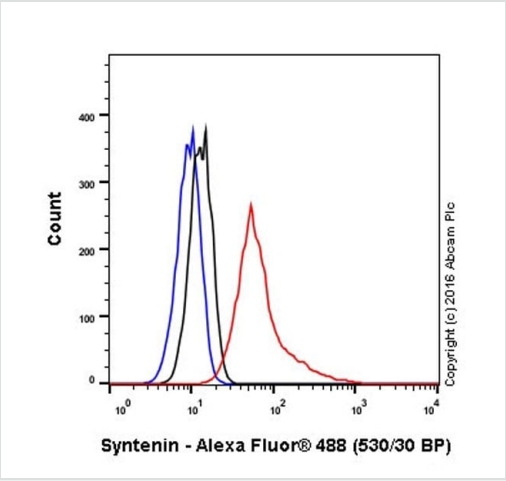
Secondary

Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution (Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated)

Predicted band size: 32 kDa

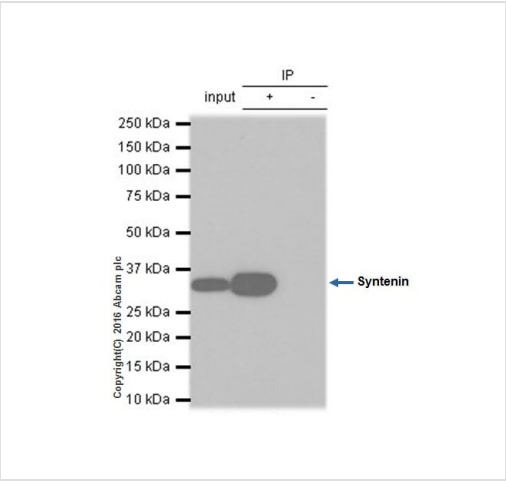
Observed band size: 32 kDa

Blocking and dilution buffer: 5% NFDM/TBST



Flow Cytometry (Intracellular) - Anti-Syntenin antibody [EPR8102] (ab133267)

Intracellular Flow Cytometry analysis ofHeLa (Human epithelial cell line from cervix adenocarcinoma)labeling Syntenin with purifiedab133267 at 1/50 (red).Cells were fixed with 4% paraformaldehyde. A goat anti rabbit IgG (Alexa Fluor® 488) 1/2000 was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Immunoprecipitation - Anti-Syntenin antibody [EPR8102] (ab133267)

ab133267 (purified) at 1/40 immunoprecipitating Syntenin in HeLa whole cell lysate.

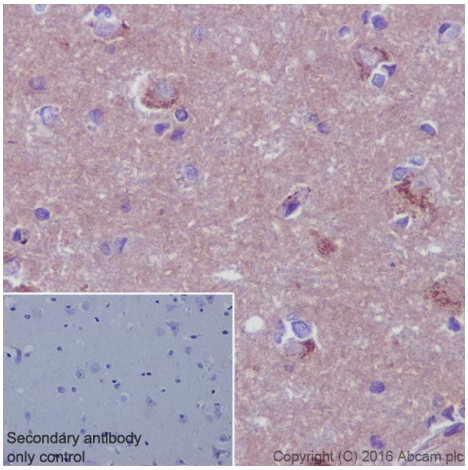
Lane 1 (input): HeLa whole cell lysate (10µg)

Lane 2 (+): ab133267 + HeLa whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab133267 in HeLa whole cell lysate.

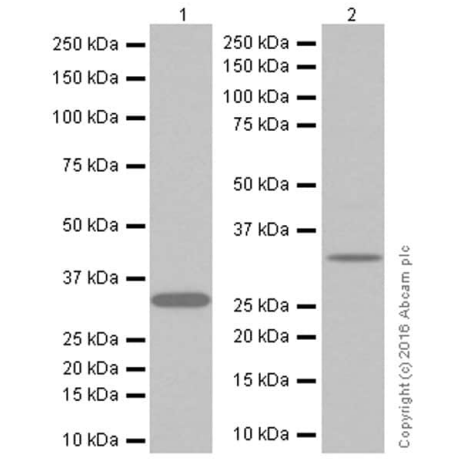
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Syntenin antibody [EPR8102] (ab133267)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human cerebral cortex tissue labeling Syntenin with purified ab133267 at 1/50 dilution. Heat mediated antigen retrieval was performed using EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Western blot - Anti-Syntenin antibody [EPR8102] (ab133267)

All lanes : Anti-Syntenin antibody [EPR8102] (ab133267) at 1/2000 dilution (purified)

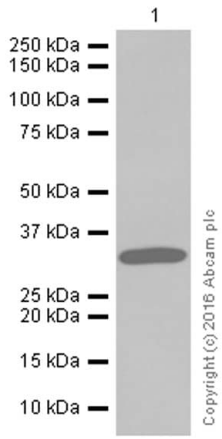
Lane 1 : Human placenta lysate
Lane 2 : Human fetal brain lysate

Lysates/proteins at 15 µg per lane.

Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

Predicted band size: 32 kDa
Observed band size: 32 kDa

Blocking and dilution buffer: 5% NFDM/TBST



Western blot - Anti-Syntenin antibody [EPR8102] (ab133267)

Anti-Syntenin antibody [EPR8102] (ab133267) at 1/10000 dilution (purified) + HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

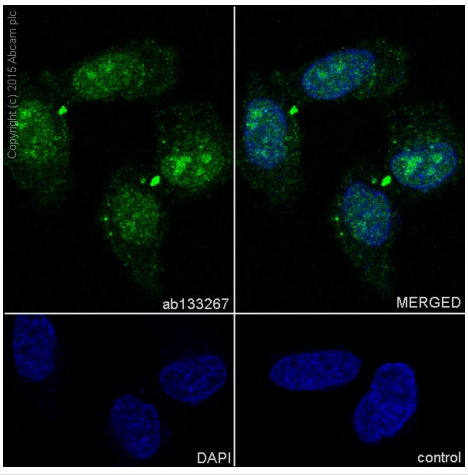
Secondary

Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/2000 dilution

Predicted band size: 32 kDa

Observed band size: 32 kDa

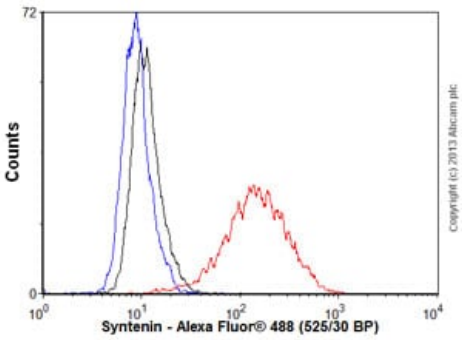
Blocking and dilution buffer: 5% NFDM/TBST



Immunocytochemistry/ Immunofluorescence - Anti-Syntenin antibody [EPR8102] (ab133267)

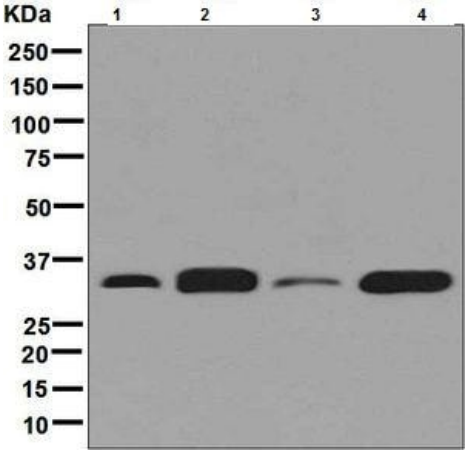
ab133267 staining Syntenin in HeLa (human cervix adenocarcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/500. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a dilution of 1/1000. DAPI was used as a nuclear counterstain.

Negative control 1: PBS only.



Flow Cytometry (Intracellular) - Anti-Syntenin antibody [EPR8102] (ab133267)

Overlay histogram showing SHSY-5Y cells stained with unpurified ab133267 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab133267, 1/10000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This antibody gave a positive signal in SHSY-5Y cells fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Western blot - Anti-Syntenin antibody [EPR8102] (ab133267)

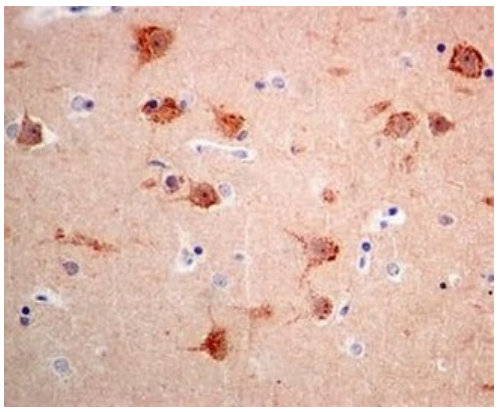
All lanes : Anti-Syntenin antibody [EPR8102] (ab133267) at 1/1000 dilution (unpurified)

Lane 1 : Human fetal brain lysate
Lane 2 : 293T cell lysate
Lane 3 : Human fetal heart lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat anti-rabbit HRP conjugated antibody at 1/2000 dilution

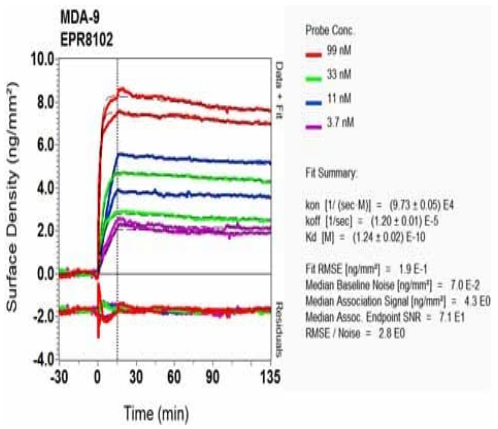
Predicted band size: 32 kDa
Observed band size: 32 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Syntenin antibody [EPR8102] (ab133267)

Immunohistochemical analysis of Syntenin in paraffin embedded Human brain tissue, using unpurified ab133267 at a 1/50 dilution.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



OI-RD Scanning - Anti-Syntenin antibody [EPR8102] (ab133267)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

Anti-Syntenin antibody [EPR8102] (ab133267)

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