

Anti-Flotillin 1 antibody [EPR6041] - BSA and Azide free ab232405

KO VALIDATED

Recombinant

RabMAb

8 Images

Overview

Product name	Anti-Flotillin 1 antibody [EPR6041] - BSA and Azide free
Description	Rabbit monoclonal [EPR6041] to Flotillin 1 - BSA and Azide free
Host species	Rabbit
Tested applications	Suitable for: WB, ICC/IF, IP, IHC-P, Flow Cyt (Intra)
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HEK293T, HAP1 and HeLa cell lysates. IP: K562 cell lysate. Flow Cyt (intra): Jurkat cells. IHC-P: Human cervical carcinoma and colon tissue. ICC/IF: Jurkat cells.
General notes	<p>ab232405 is the carrier-free version of ab133497.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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	EPR6041
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab232405 in the following tested applications.

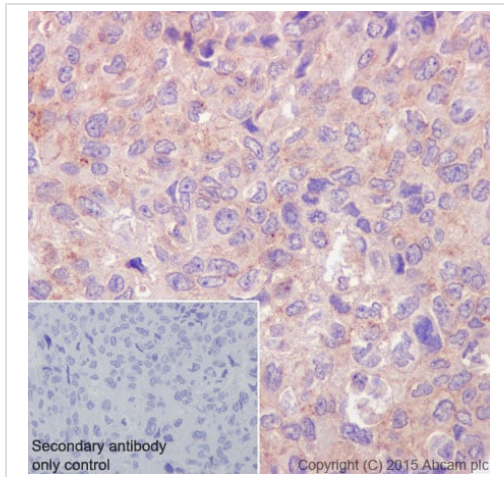
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 47 kDa.
ICC/IF		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Target

Function	May act as a scaffolding protein within caveolar membranes, functionally participating in formation of caveolae or caveolae-like vesicles.
Sequence similarities	Belongs to the band 7/mec-2 family. Flotillin subfamily.
Cellular localization	Cell membrane. Membrane > caveola. Melanosome. Endosome. Membrane-associated protein of caveolae. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

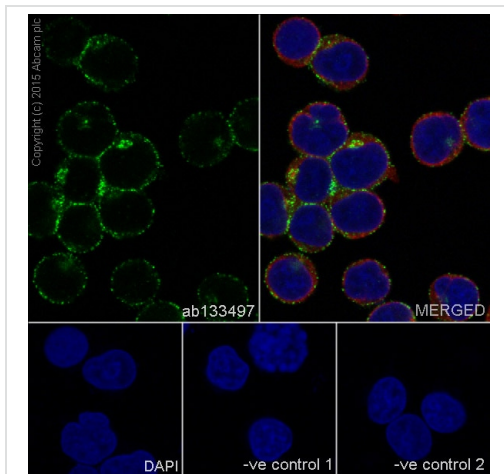
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Flotillin 1 antibody [EPR6041] - BSA and Azide free (ab232405)

Immunohistochemical staining of paraffin embedded human cervical carcinoma with purified **ab133497** at a working dilution of 1/100. The secondary antibody used is HRP goat anti-rabbit IgG H&L (**ab97051**) at 1/500. The sample is counter-stained with hematoxylin. Antigen retrieval was performed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.

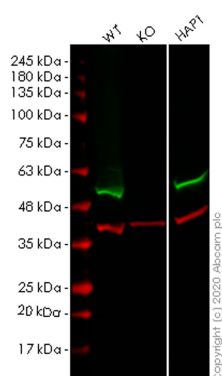
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab133497**).



Immunocytochemistry/ Immunofluorescence - Anti-Flotillin 1 antibody [EPR6041] - BSA and Azide free (ab232405)

Immunofluorescence staining of Jurkat cells with purified **ab133497** at a working dilution of 1/500, counter-stained with DAPI. The secondary antibody was Alexa Fluor® 488 goat anti-rabbit (**ab150077**), used at a dilution of 1/1000. **ab7291**, a mouse anti-tubulin antibody (1/1000), was used to stain tubulin along with **ab150120** (Alexa Fluor® 594 goat anti-mouse, 1/1000), shown in the top right hand panel. The cells were fixed in 4% PFA and permeabilized using 0.1% Triton X 100. The negative controls are shown in bottom middle and right hand panels - for negative control 1, purified **ab133497** was used at a dilution of 1/500 followed by an Alexa Fluor® 594 goat anti-mouse antibody (**ab150120**) at a dilution of 1/500. For negative control 2, **ab7291** (mouse anti-tubulin) was used at a dilution of 1/500 followed by an Alexa Fluor® 488 goat anti-rabbit antibody (**ab150077**) at a dilution of 1/400.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab133497**).



Western blot - Anti-Flotillin 1 antibody [EPR6041] - BSA and Azide free (ab232405)

All lanes : Anti-Flotillin 1 antibody [EPR6041] ([ab133497](#)) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate

Lane 2 : FLOT1 knockout HEK293T cell lysate

Lane 3 : HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed ([ab216773](#)) at 1/10000 dilution

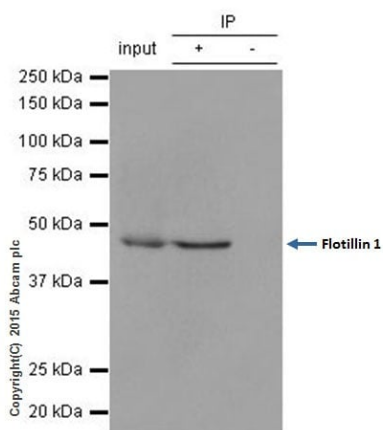
Predicted band size: 47 kDa

Observed band size: 50 kDa

This data was developed using the same antibody clone in a different buffer formulation ([ab133497](#)).

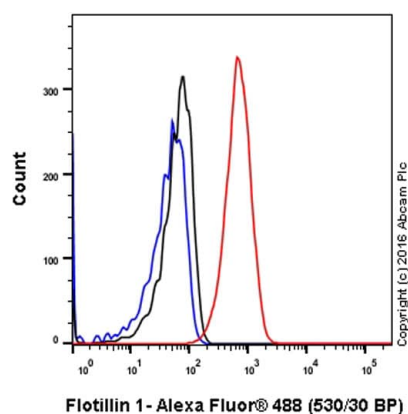
Lanes 1-3: Merged signal (red and green). Green - [ab133497](#) observed at 50 kDa. Red - loading control [ab8245](#) observed at 36 kDa.

[ab133497](#) Anti-Flotillin 1 antibody [EPR6041] was shown to specifically react with Flotillin 1 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line [ab267276](#) (knockout cell lysate [ab257109](#)) was used. Wild-type and Flotillin 1 knockout samples were subjected to SDS-PAGE. [ab133497](#) 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.



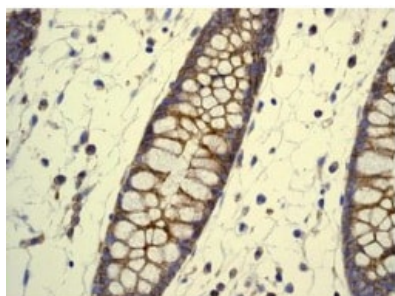
Immunoprecipitation - Anti-Flotillin 1 antibody
[EPR6041] - BSA and Azide free (ab232405)

ab133497 (purified) at 1/60 immunoprecipitating Flotillin 1 in 10 µg K562 (Lanes 1 and 2, observed at 48 kDa). Lane 3 - PBS. For western blotting, a HRP-conjugated anti-rabbit IgG, specific to the non-reduced form of IgG was used as the secondary antibody (1/1500). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab133497**).



Flow Cytometry (Intracellular) - Anti-Flotillin 1
antibody [EPR6041] - BSA and Azide free
(ab232405)

Intracellular Flow Cytometry analysis of Jurkat (human acute T cell leukemia) cells labeling Flotillin 1 with purified **ab133497** at 1/50 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor®488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab133497**).

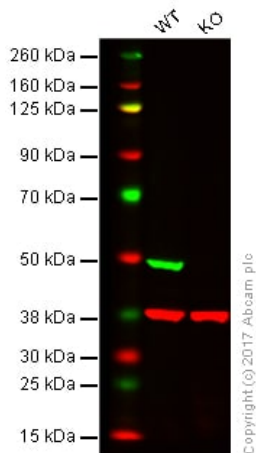


Immunohistochemistry (Formalin/PFA-fixed paraffin-
embedded sections) - Anti-Flotillin 1 antibody
[EPR6041] - BSA and Azide free (ab232405)

Immunohistochemical analysis of paraffin embedded Human colon tissue labelling Flotillin 1 using unpurified **ab133497** at a dilution of 1/100.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab133497**).

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



Western blot - Anti-Flotillin 1 antibody [EPR6041] - BSA and Azide free (ab232405)

Lane 1: Wild-type HAP1 whole cell lysate (20 µg)

Lane 2: Flotillin 1 knockout HAP1 whole cell lysate (20 µg)

Lanes 1 - 2: Merged signal (red and green). Green - [ab133497](#) observed at 47 kDa. Red - loading control, [ab9484](#), observed at 37 kDa.

[ab133497](#) was shown to specifically react with Flotillin 1 in wild-type HAP1 cells as signal was lost in Flotillin 1 knockout cells. Wild-type and Flotillin 1 knockout samples were subjected to SDS-PAGE.

[ab133497](#) and [ab9484](#) (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10,000 dilution and 1/20,000 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 1/20,000 dilution for 1 hour at room temperature before imaging.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide ([ab133497](#)).

Why choose a recombinant antibody?



Anti-Flotillin 1 antibody [EPR6041] - BSA and Azide free (ab232405)

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