abcam

Product datasheet

Anti-Flotillin 1 antibody [EPR6041] ab133497





重组 RabMAb

★★★★★ 5 Abreviews 51 References 11 图像

概述

产品名称 Anti-Flotillin 1抗体[EPR6041]

描述 兔单克隆抗体[EPR6041] to Flotillin 1

宿主 Rabbit

经测试应用 适用于: Flow Cyt (Intra), WB, IHC-P, IP, ICC/IF

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide within Human Flotillin 1 aa 400-500 (C terminal). The exact sequence is

proprietary.

Database link: **O75955**

阳性对照 WB: HEK293T, HAP1, K562, A431, Jurkat, and HeLa cell lysates; Mouse brain and kidney

lysates; Rat kidney lysate. IP: K562 cell lysate. IHC-P: Human cervical carcinoma tissue. Flow Cyt

(intra): Jurkat cells. ICC/IF: Jurkat cells.

常规说明 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**.

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Stable for 12 months at -20°C.

存储溶液 pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

纯度 Protein A purified

克隆 单克隆

克隆编号 EPR6041

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab133497于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
Flow Cyt (Intra)		1/50.
WB	*** <u>*</u>	1/10000 - 1/50000. Detects a band of approximately 48 kDa (predicted molecular weight: 47 kDa).
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
IP		1/10 - 1/100.
ICC/IF		1/500.

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功能 May act as a scaffolding protein within caveolar membranes, functionally participating in formation

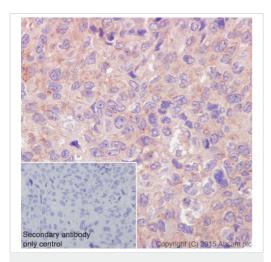
of caveolae or caveolae-like vesicles.

序列相似性 Belongs to the band 7/mec-2 family. Flotillin subfamily.

细胞定位 Cell membrane > caveola. Melanosome. Endosome. Membrane-associated protein

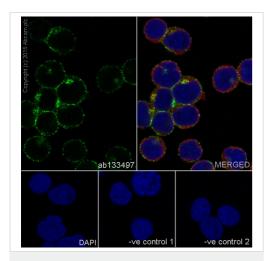
of caveolae. Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

图片



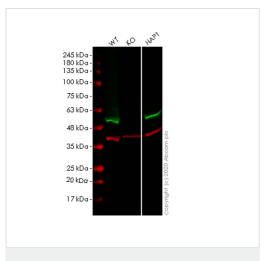
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Flotillin 1 antibody
[EPR6041] (ab133497)

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 perfomed 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.



Immunocytochemistry/ Immunofluorescence - Anti-Flotillin 1 antibody [EPR6041] (ab133497)

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 antitubulin 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 antitubulin) 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.



Western blot - Anti-Flotillin 1 antibody [EPR6041] (ab133497)

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.

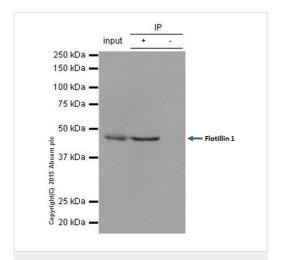
Secondary

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

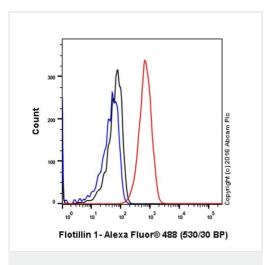
Predicted band size: 47 kDa Observed band size: 50 kDa

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 <u>ab267276</u> (knockout cell lysate <u>ab257109</u>) was used. Wild-type and Flotillin 1 knockout samples were subjected to SDS-PAGE. ab133497 and Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

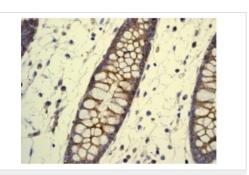


Immunoprecipitation - Anti-Flotillin 1 antibody [EPR6041] (ab133497) ab133497 (purified) at 1/60 immunoprecipitating Flotillin 1 in 10 μ g K562 (Lanes 1 and 2, observed at 48 kDa). Lane 3 - PBS. For western blotting, a HRP-conjugated anti-rabbit lgG, specific to the non-reduced form of lgG was used as the secondary antibody (1/1500). Blocking buffer and concentration: 5% NFDM/TBST Dilution buffer and concentration: 5% NFDM/TBST



Flow Cytometry (Intracellular) - Anti-Flotillin 1 antibody [EPR6041] (ab133497)

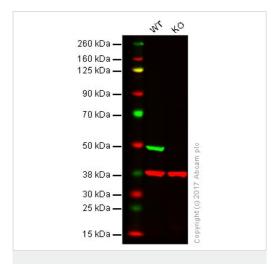
Intracellular Flow Cytometry analysis of Jurkat (human acute T cell leukemia) cells labeling Flotillin 1 with purified ab133497 at 1/50 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluorr[®]488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Flotillin 1 antibody
[EPR6041] (ab133497)

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

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



Western blot - Anti-Flotillin 1 antibody [EPR6041] (ab133497)

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 <u>ab9484</u> (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/10000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



(ab133497)

All lanes: Anti-Flotillin 1 antibody [EPR6041] (ab133497) at

1/10000 dilution (purified)

Lane 1: K562 cell lysate Lane 2: A431 cell lysate Lane 3: Jurkat cell lysate Lane 4: HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: HRP goat anti-rabbit lgG (H+L) at 1/1000 dilution

Predicted band size: 47 kDa Observed band size: 48 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST

All lanes: Anti-Flotillin 1 antibody [EPR6041] (ab133497) at 1/10000 dilution (purified)

Lane 1: mouse brain lysate Lane 2: mouse kidney lysate Lane 3: rat kidney lysate

Lysates/proteins at 20 µg per lane.

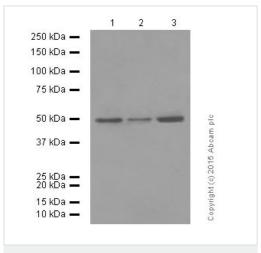
Secondary

All lanes: HRP goat anti-rabbit lgG (H+L)

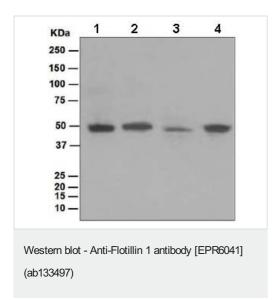
Predicted band size: 47 kDa Observed band size: 48 kDa

Blocking buffer: 5% NFDM/TBST

Dilution buffer: 5% NFDM/TBST



Western blot - Anti-Flotillin 1 antibody [EPR6041] (ab133497)



All lanes : Anti-Flotillin 1 antibody [EPR6041] (ab133497) at 1/10000 dilution

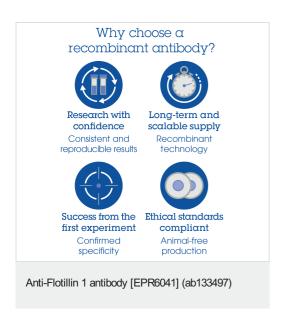
Lane 1 : K562 cell lysate Lane 2 : A431 cell lysate Lane 3 : Jurkat cell lysate Lane 4 : HeLa cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes: HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 47 kDa
Observed band size: 48 kDa



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