abcam

Product datasheet

Anti-VAMP8/EDB antibody [EP2629Y] ab76021

敲除验证 重组 RabMAb

★★★★★ 1 Abreviews 28 References 12 图像

概述

产品名称	Anti-VAMP8/EDB抗体[EP2629Y]
描述	兔单克隆抗体[EP2629Y] to VAMP8/EDB
宿主	Rabbit
经测试应用	**适用于:** Flow Cyt (Intra), WB, IP, IHC-P, ICC/IF
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: A431, 293T, HEK-293, HeLa, RAW264.7, NIH/3T3 and PC-12 cell lysates; Mouse kidney tissue lysate. IHC-P: Human brain and kidney tissue. ICC/IF: PC-12 cells. Flow Cyt (intra): HeLa cells. IP: HEK293 cell lysate.
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. Avoid freeze / thaw cycle.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
纯度	Protein A purified
克隆	单克隆
克隆编号	EP2629Y

同种型 IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab76021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)		1/80 - 1/150. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★★ (1)	1/10000 - 1/20000. Detects a band of approximately 17 kDa (predicted molecular weight: 11 kDa).
IP		1/20 - 1/50.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**.
ICC/IF		1/100 - 1/250.

靶标

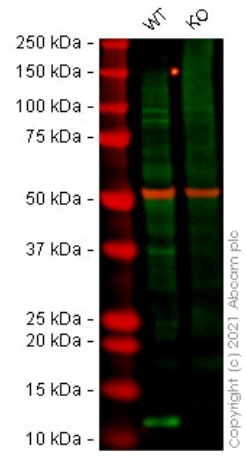
功能 Involved in the targeting and/or fusion of transport vesicles to their target membrane. Involved for dense-granule secretion in platelets. Plays a role in regulated enzyme secretion in pancreatic acinar cells. Involved in the abscission of the midbody during cell division, which leads to completely separate daughter cells. Involved in the homotypic fusion of early and late endosomes.

组织特异性 Platelets.

序列相似性 Belongs to the synaptobrevin family.
Contains 1 v-SNARE coiled-coil homology domain.

细胞定位 Membrane.

图片



Western blot - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

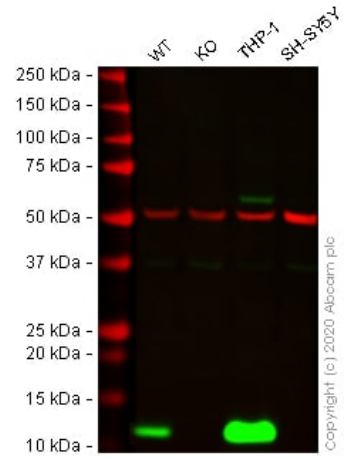
All lanes : Anti-VAMP8/EDB antibody [EP2629Y] (ab76021) at 1/10000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : VAMP8 knockout HEK-293T cell lysate

Performed under reducing conditions.

Predicted band size: 11 kDa
Observed band size: 11 kDa

False colour image of Western blot: Anti-VAMP8/EDB antibody [EP2629Y] staining at 1/10000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab76021 was shown to bind specifically to VAMP8/EDB. A band was observed at 11 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in VAMP8 knockout cell line **ab266293** (knockout cell lysate **ab257791**). To generate this image, wild-type and VAMP8 knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4°C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged.Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

All lanes : Anti-VAMP8/EDB antibody [EP2629Y] (ab76021) at 1/10000 dilution

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 2 : VAMP8 knockout A-431 (Human epidermoid carcinoma cell line) whole cell lysate
Lane 3 : THP-1 (Human monocytic leukemia cell line) whole cell lysate
Lane 4 : SH-SY5Y (Human neuroblastoma cell line from bone marrow) whole cell lysate

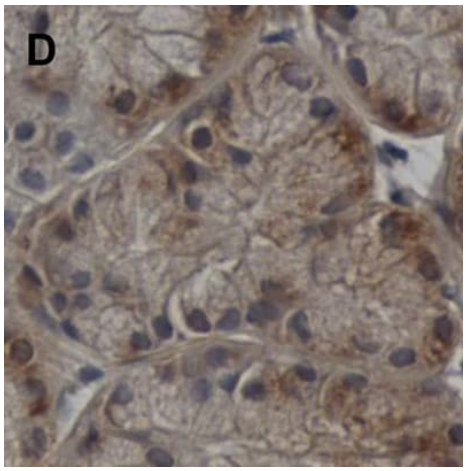
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 11 kDa
Observed band size: 13 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab76021 observed at 13 kDa. Red - loading control, **ab7291** (Mouse anti-Alpha Tubulin [DM1A] observed at 55kDa.

ab76021 was shown to react with VAMP8/EDB in wild-type A431 cells in western blot. Loss of signal was observed when VAMP8 knockout sample was used. Wild-type and VAMP8 knockout A431 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween®) before incubation with ab76021 and **ab7291** (Mouse anti-Alpha Tubulin [DM1A] overnight at 4°C at a 1 in 10000 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.

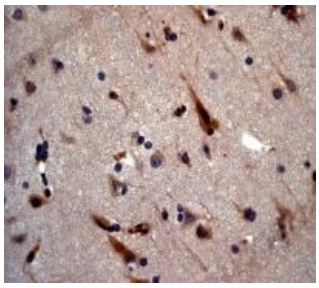


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

Image from Kamoi M et al., PLoS One. 2012;7(9):e43688. doi: 10.1371/journal.pone.0043688. Epub 2012 Sep 4. Fig 4.; doi:10.1371/journal.pone.0043688; September 4, 2012, PLoS ONE 7(9): e43688.

Immunohistochemical analysis of Human lacrimal gland tissue staining VAMP8/EDB with unpurified ab76021.

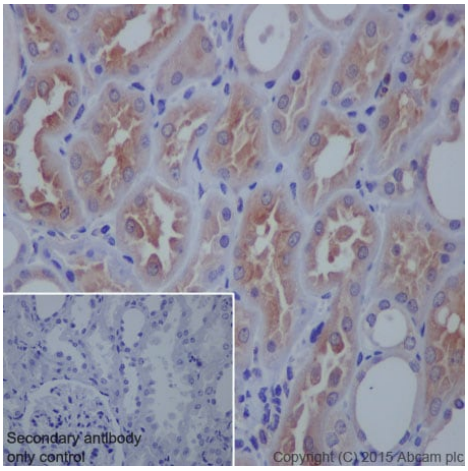
Antigen retrieval was performed using antigen retrieval solution in a microwave. Sections were blocked with 10 goat serum for 30 minutes and incubated with primary antibody (1/100) overnight at 4°C. Staining was detected using DAB.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

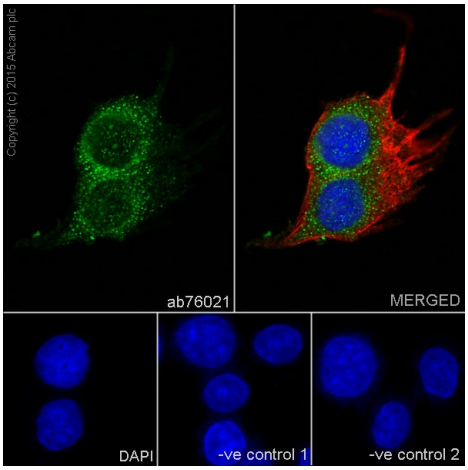
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human brain tissue labelling VAMP8/EDB with unpurified ab76021 at a dilution of 1/100. A HRP/AP polymerized secondary antibody was used.

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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human kidney tissue labelling VAMP8/EDB with purified ab76021 at a dilution of 1/250. 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.

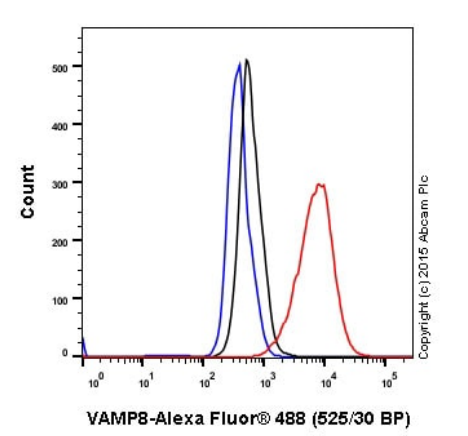


Immunocytochemistry/ Immunofluorescence - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

Immunocytochemistry/Immunofluorescence analysis of PC-12 cells labelling VAMP8/EDB with purified ab76021 at a dilution of 1/100. Cells were fixed with 100% methanol 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. DAPI (blue) was used as the nuclear counterstain. **ab7291**, a mouse anti-tubulin (1/1000) and **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000) were also used.

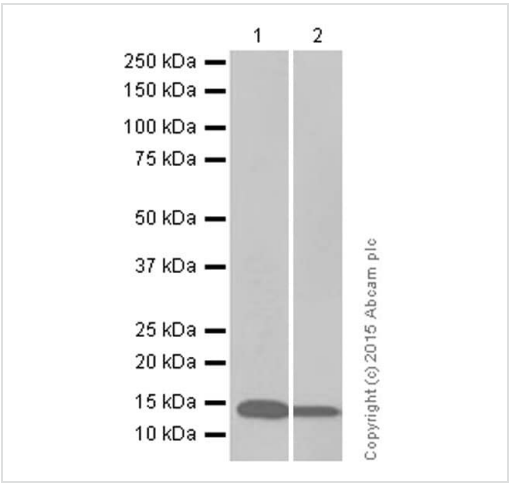
Control 1: primary antibody (1/100) and secondary antibody, **ab150120**, an Alexa Fluor® 594-conjugated goat anti-mouse IgG (1/1000).

Control 2: **ab7291** (1/1000) and secondary antibody, **ab150077**, an Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/1000).



Flow Cytometry (Intracellular) - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

Intracellular Flow Cytometry analysis of HeLa cells labelling VAMP8/EDB with purified ab76021 at a dilution of 1/150 (red). Cells were fixed with 4% paraformaldehyde. An Alexa Fluorr® 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



Western blot - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

All lanes : Anti-VAMP8/EDB antibody [EP2629Y] (ab76021) at 1/10000 dilution (purified)

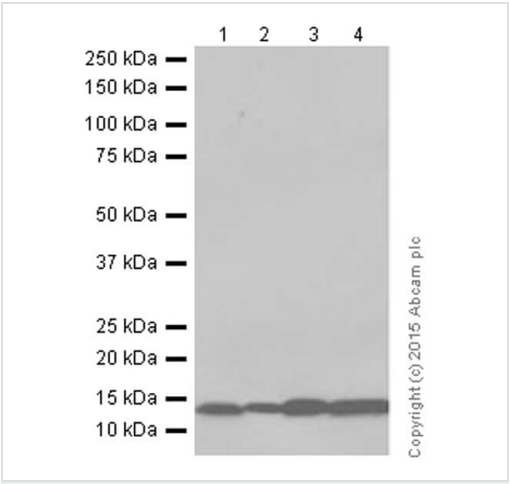
Lane 1 : HEK293 whole cell lysate
Lane 2 : HeLa whole cell lysate

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: 11 kDa
Observed band size: 15 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Western blot - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

All lanes : Anti-VAMP8/EDB antibody [EP2629Y] (ab76021) at 1/10000 dilution (purified)

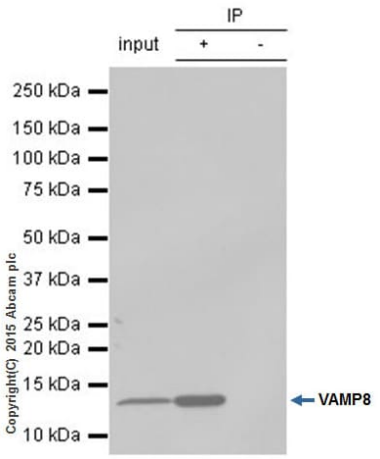
Lane 1 : Rat kidney tissue lysate
Lane 2 : Mouse kidney tissue lysate
Lane 3 : RAW264.7 whole cell lysate
Lane 4 : NIH/3T3 whole cell lysate

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: 11 kDa
Observed band size: 15 kDa

Blocking and dilution buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

ab76021 (purified) at 1/50 immunoprecipitating VAMP8/EDB in HEK293 whole cell lysate.

Lane 1 (input): HEK293 whole cell lysate (10µg)

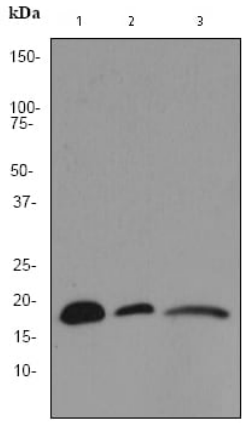
Lane 2 (+): ab76021 + HEK293 whole cell lysate.

Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab76021 in HEK293 whole cell lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10,000 dilution.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.



Western blot - Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

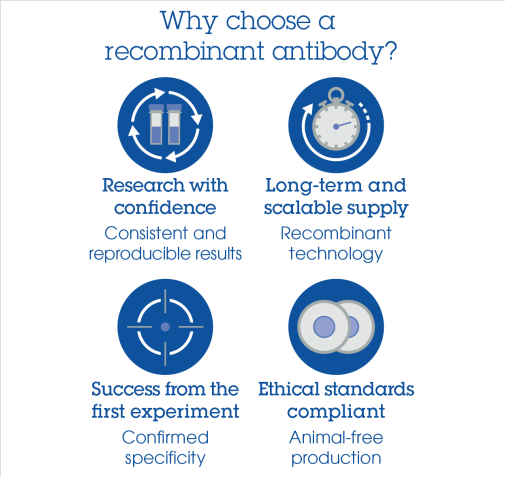
All lanes : Anti-VAMP8/EDB antibody [EP2629Y] (ab76021) at 1/20000 dilution (unpurified)

Lane 1 : 293T cell lysate
Lane 2 : HeLa cell lysate
Lane 3 : PC12 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-conjugated goat anti-rabbit IgG at 1/2000 dilution

Predicted band size: 11 kDa
Observed band size: 17 kDa



Anti-VAMP8/EDB antibody [EP2629Y] (ab76021)

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