abcam

Product datasheet

Anti-VPS35 antibody [2D3] ab57632

敲除验证

18 References 4 图像

概述

产品名称	Anti-VPS35抗体[2D3]
描述	**小鼠单克隆抗体**[2D3] to VPS35
宿主	Mouse
经测试应用	**适用于:** WB, ICC/IF, Flow Cyt
种属反应性	**与反应:** Human, Recombinant fragment
免疫原	Recombinant fragment: MECLKKALKI ANQCMDPSLQ VQLFIEILNR YIYFYEKEND AVTIQVLNQL IQKIREDLPN LESSEETEQI NKHFHNTLEH LRLRRESPES EGPIYEGLIL , corresponding to amino acids 697-797 of Human VPS35 Run BLAST with ExPASy Run BLAST with NCBI
阳性对照	Flow Cyt: HEK293 cells. WB: Wild-type HAP1, NIH/3T3, A549 cell lysates, tagged recombinant protein. ICC/IF: Wild-type HAP1 cells.
常规说明	This product was changed from ascites to tissue culture supernatant on 05 Feb 2019. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team. The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing. If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As

性能

形式	Liquid
存放说明	Shipped at 4°C. Upon delivery aliquot and store at -20°C or -80°C. Avoid repeated freeze / thaw cycles.
存储溶液	pH: 7.4
纯度	Tissue culture supernatant

纯化说明	Purified from TCS.
克隆	单克隆
克隆编号	2D3
同种型	IgG2a
轻链类型	kappa

应用

The Abpromise guarantee Abpromise™承诺保证使用ab57632于以下的经测试应用

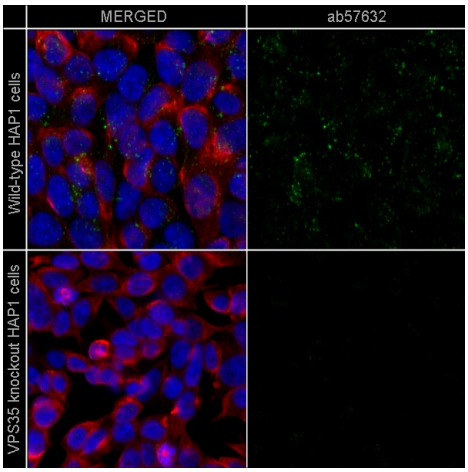
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB		Use at an assay dependent concentration. This antibody has only been tested in WB against the recombinant fragment used as immunogen. We have no data on the detection of endogenous protein.
ICC/IF		Use a concentration of 0.2 µg/ml.
Flow Cyt		Use at an assay dependent concentration. **ab170191** - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.

靶标

功能	Essential component of the retromer complex, a complex required to retrieve lysosomal enzyme receptors (IGF2R and M6PR) from endosomes to the trans-Golgi network. Also required to regulate transcytosis of the polymeric immunoglobulin receptor (pIgR-pIgA).
组织特异性	Ubiquitous. Highly expressed in heart, brain, placenta, skeletal muscle, spleen, thymus, testis, ovary, small intestine, kidney and colon.
序列相似性	Belongs to the VPS35 family.
细胞定位	Cytoplasm. Membrane.

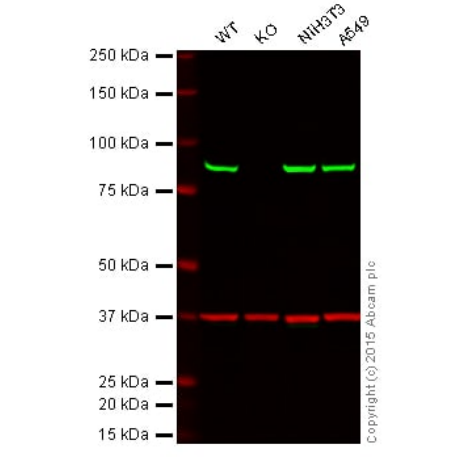
图片



Immunocytochemistry/ Immunofluorescence - Anti-VPS35 antibody [2D3] (ab57632)

Ab57632 staining VPS35 in wild-type HAP1 cells (top panel) and VPS35 knockout HAP1 cells (bottom panel). The cells were fixed with 4% PFA (10 min), 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 ab57632 at 0.2 µg/mL and **ab6046** at 1 µg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Mouse IgG (Alexa Fluor® 488) (**ab150117**) (shown in pseudo colour green) and goat secondary antibody to Rabbit IgG (Alexa Fluor® 594) (**ab150084**) (shown in pseudo colour red) both at 1/1000. Nuclear DNA was labelled with DAPI (shown in pseudo colour blue).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



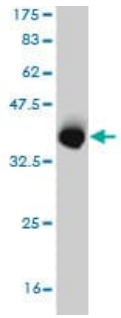
Western blot - Anti-VPS35 antibody [2D3] (ab57632)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: VPS35 knockout HAP1 cell lysate (20 µg)
Lane 3: NIH/3T3 cell lysate (20 µg)
Lane 4: A549 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab57632 observed at 91 kDa. Red - loading control, **ab181602**, observed at 37 kDa.

ab57632 was shown to specifically react with VPS35 in wild-type HAP1 cells. No band was observed when VPS35 knockout samples were examined. Wild-type and VPS35 knockout samples were subjected to SDS-PAGE. **ab5732** and **ab181602** (loading control to GAPDH) were diluted 1µg/mL and 1/10,000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (**ab216772**) and Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (**ab216777**) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.

This image was generated using a version of the antibody

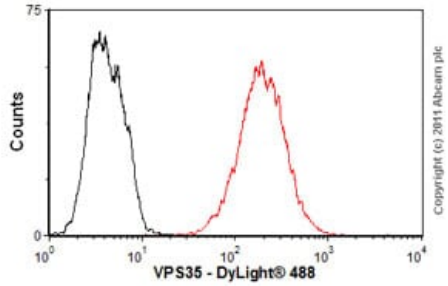
produced in ascites.



Western blot - Anti-VPS35 antibody [2D3] (ab57632)

Western blot against tagged recombinant protein immunogen using ab57632 VPS35 antibody at 1 µg/ml. Predicted band size of immunogen is 37 kDa

This image was generated using a version of the antibody produced in ascites.



Flow Cytometry - Anti-VPS35 antibody [2D3] (ab57632)

Overlay histogram showing HEK293 cells stained with ab57632 (red line). The cells were fixed with 80% methanol (5 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 (ab57632, 0.5µg/1x10^6 cells) for 30 min at 22ºC. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22ºC. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (**ab91361**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in HEK293 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

This image was generated using a version of the antibody produced in ascites.

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