abcam

Product datasheet

Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] ab14734

敲除验证

★★★★★ 52 Abreviews 512 References 12 图像

概述

产品名称	Anti-VDAC1/Porin + VDAC3抗体[20B12AF2]
描述	小鼠单克隆抗体[20B12AF2] to VDAC1/Porin + VDAC3
宿主	Mouse
经测试应用	**适用于:** WB, ICC/IF, Flow Cyt
种属反应性	**与反应:** Mouse, Rat, Cow, Human **预测可用于:** Sheep, Goat, Cat, Dog, Pig, Drosophila melanogaster, Fish, Quail, Common marmoset, Dogfish, Catshark
免疫原	Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: Isolated mitochondria from human, cow, rat and mouse heart. HepG2 cell lysate. ICC/IF: HeLa cells. Human fibroblasts. Flow Cyt: HepG2 cells.
常规说明	This antibody clone [20B12AF2] is manufactured by Abcam. If you require this antibody in a different buffer formulation or a different conjugate for your experiments, please contact **orders@abcam.com** or you can find further information **here**. 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. Store at +4°C. Do Not Freeze.
存储溶液	pH: 7.50 Preservative: 0.02% Sodium azide Constituents: 0.36% HEPES, 0.88% Sodium chloride
纯度	IgG fraction

纯化说明	Near homogeneity as judged by SDS-PAGE. The antibody was produced in vitro using hybridomas grown in serum-free medium, and then purified by biochemical fractionation.
克隆	单克隆
克隆编号	20B12AF2
同种型	IgG2b
轻链类型	kappa

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab14734于以下的经测试应用**

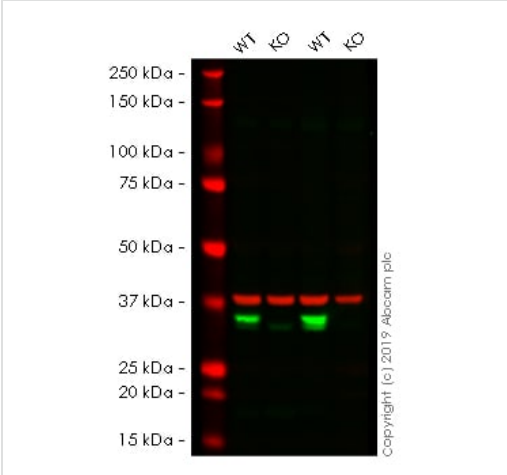
"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
WB	★★★★★ (18)	Use a concentration of 1 µg/ml. Detects a band of approximately 39 kDa.
ICC/IF	★★★★★ (4)	Use at an assay dependent concentration.
Flow Cyt		Use 1µg for 10^6 cells. **ab170192** - Mouse monoclonal IgG2b, is suitable for use as an isotype control with this antibody.

靶标

细胞定位 VDAC1/Porin: Mitochondrion outer membrane. Cell membrane. VDAC3: Mitochondrial outer membrane.

图片



Western blot - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

All lanes : Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734) at 1/1000 dilution

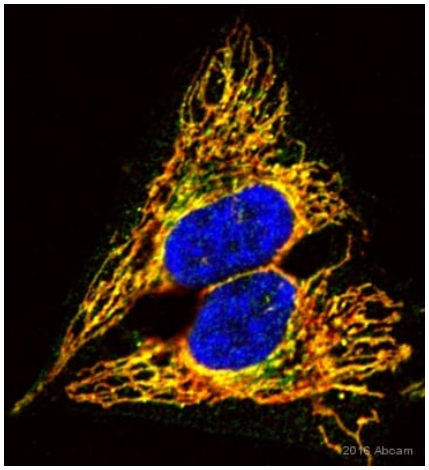
Lane 1 : Wild-type Hap1 cell lysate
Lane 2 : VDAC1 knockout Hap1 cell lysate
Lane 3 : Wild-type HEK-293T cell lysate
Lane 4 : VDAC1 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Lanes 1 - 4: Merged signal (red and green). Green - ab14734 observed at 31 kDa. Red - loading control, **ab181602** observed at 37 kDa. The lower band very close to VDAC1 is likely to be

VDAC3.

ab14734 was shown to react with VDAC1/Porin + VDAC3 in wild-type HEK-293T cells. Loss of signal was observed when knockout cell line **ab255444** (knockout cell lysate **ab263839**) was used. Wild-type and VDAC1 / Porin knockout samples were subjected to SDS-PAGE. ab14734 and Anti-GAPDH antibody [EPR16891] - Loading Control (**ab181602**) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

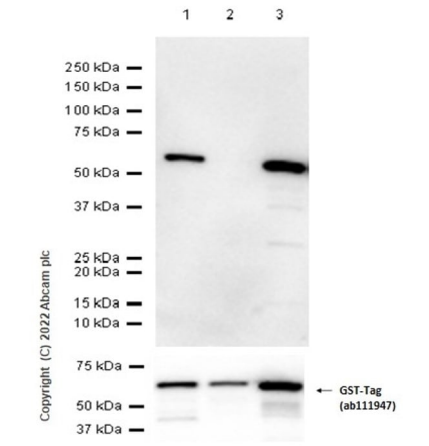


Immunocytochemistry/ Immunofluorescence - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

This image is courtesy of an Abreview by Michiel Krols.

ab14734 staining VDAC1/Porin + VDAC3 in human HeLa (Human epithelial cell line from cervix adenocarcinoma) cells by ICC/IF (Immunocytochemistry/immunofluorescence).

Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100 for 3 minutes and blocked with 0.2% serum for 60 minutes at 22°C. Samples were incubated with primary antibody (1/200 in 0.5% BSA and 0.02% Triton X100 in PBS) for 16 hours at 4°C. An FITC-conjugated goat anti-mouse IgG polyclonal was used as the secondary antibody at a dilution of 1/200.



Western blot - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

All lanes : Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734) at 1/1000 dilution

Lane 1 : N-GST tagged Recombinant Human VDAC1 (aa1 to 283) protein with NFDM/TBST
Lane 2 : N-GST tagged Recombinant Human VDAC2 (aa 1 to 294) protein with NFDM/TBST
Lane 3 : N-GST tagged Recombinant Human VDAC3 (aa 1 to 283) protein with NFDM/TBST

Lysates/proteins at 0.01 µg per lane.

Blocking peptides at 5 % per lane.

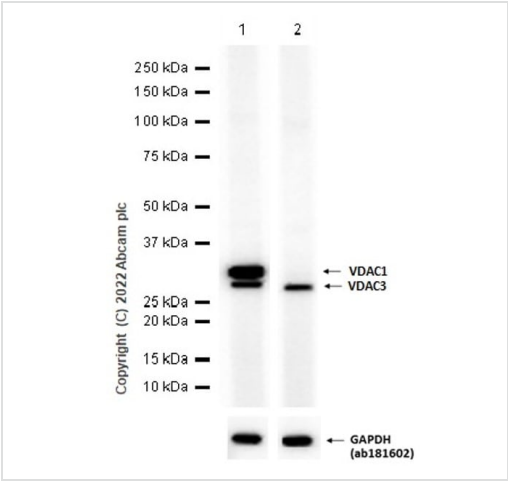
Observed band size: 33 kDa

Exposure time: 10 seconds

Diluting buffer: 5% NFDM /TBST

N-GST tagged Recombinant Human VDAC1 protein is available as **ab132481**

N-GST tagged Recombinant Human VDAC2 protein is available as **ab152793**



Western blot - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

All lanes : Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734) at 1/1000 dilution

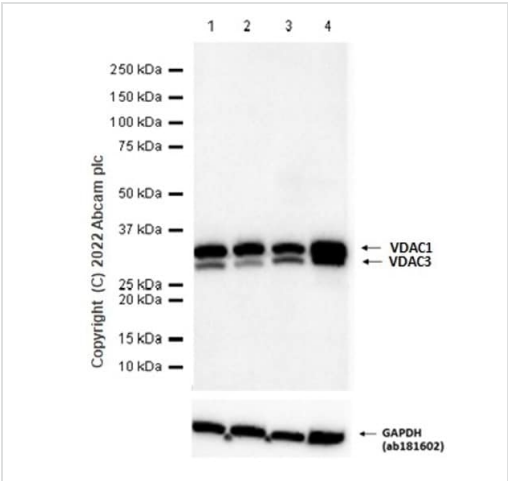
Lane 1 : Wild type HAP1 whole cell lysate
Lane 2 : VDAC1 knockout HAP1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Observed band size: 33 kDa

Exposure time: 5 seconds

Blocking and dilution buffer: 5% NFDM /TBST.



Western blot - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

All lanes : Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734) at 1/1000 dilution

Lane 1 : HEK-293 (Human embryonic kidney epithelial cell) whole cell lysate with NFDM/TBST
Lane 2 : HAP1 (Wildtype control Human chronic myelogenous leukemia near-haploid cell line) whole cell lysate with NFDM/TBST
Lane 3 : MEF (Mus musculus Embryo Fibroblast) whole cell lysate with NFDM/TBST
Lane 4 : Rat brain tissue lysate with NFDM/TBST

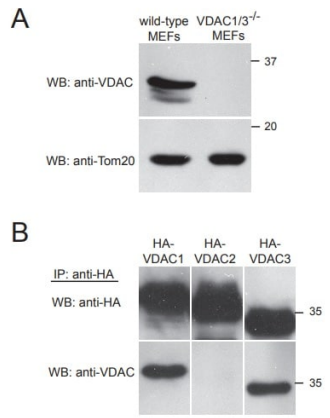
Lysates/proteins at 20 µg per lane.

Blocking peptides at 5 % per lane.

Observed band size: 33 kDa

Exposure time: 5 seconds

Diluting buffer: 5% NFDM /TBST



Western blot - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

Sun Y et al Voltage-dependent anion channels (VDACs) recruit Parkin to defective mitochondria to promote mitochondrial autophagy. J Biol Chem. 2012 Nov 23;287(48):40652-60. doi: 10.1074/jbc.M112.419721.

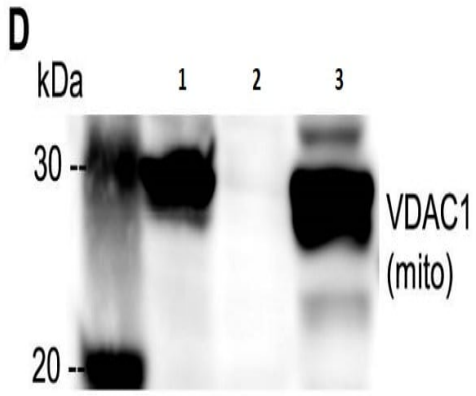
(A) The Anti-VDAC1/Porin + VDAC3 antibody recognizes VDAC1 and VDAC3 but not VDAC2.

Lysates from wild-type MEFs and VDAC1/3-/- MEFs were analyzed by western blotting with Anti-VDAC1/Porin + VDAC3 (top panel) or anti Tom20 antibodies (bottom panel).

(B) The Anti-VDAC1/Porin + VDAC3 antibody does not recognize VDAC2 in VDAC1/3-/- MEFs.

HA-tagged VDAC1, 2 or 3 were expressed in VDAC1/3-/- MEFs, immunoprecipitated with anti-HA antibodies and analyzed by western blotting with anti-HA antibodies (top panel) or Anti-VDAC1/Porin + VDAC3 antibodies (bottom panel). The Anti-VDAC1/Porin + VDAC3 antibodies recognize HA-VDAC1 and HA-VDAC3, but not HA-VDAC2 . Note that HA-VDAC3 migrates at a lower molecular weight than HA-VDAC1 and HA-VDAC2 .

Molecular weights are indicated in kDa.



Western blot - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

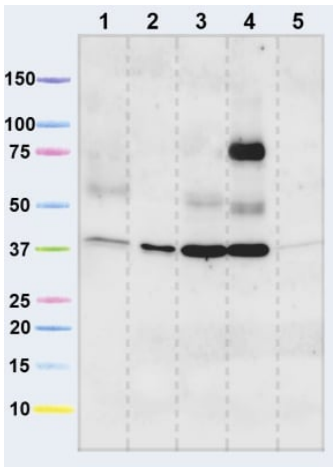
Li et al PLoS One. 2016 Feb 22;11(2):e0149728. doi: 10.1371/journal.pone.0149728. eCollection 2016. Fig 5.

Immunoblot analysis of endogenous VDAC1/Porin and VDAC3 in subcellular fractions of MA-10 cell lysates. VDAC1/Porin and VDAC3 are used as a mitochondrial marker proteins.

Lane 1: Whole cell lysate

Lane 2: Cytosolic fraction

Lane 3: Mitochondrial-enriched fraction



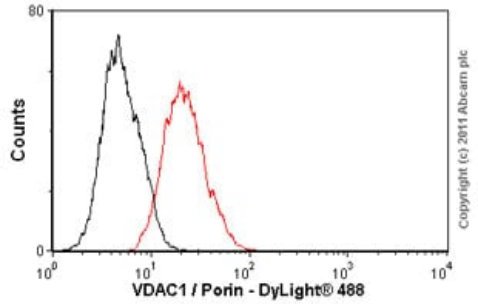
Western blot - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

All lanes : Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

Lane 1 : Isolated mitochondria from human heart at 15 µg
Lane 2 : Isolated mitochondria from bovine heart at 6 µg
Lane 3 : Isolated mitochondria from rat heart at 30 µg
Lane 4 : Isolated mitochondria from mouse heart at 30 µg
Lane 5 : HepG2 (Human liver hepatocellular carcinoma cell line) cell lysate at 30 µg

Observed band size: 37 kDa

Extra bands in the mouse sample (lane 4) are due to the reaction of the secondary antibody with residual mouse blood in the heart tissue, as it is very difficult to entirely remove the blood from these small organs.



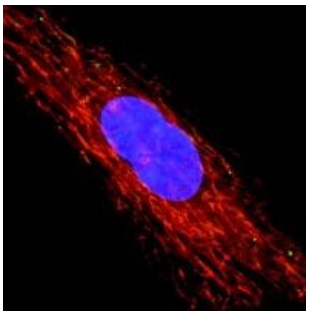
Flow Cytometry - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

Overlay histogram showing HepG2 (Human liver hepatocellular carcinoma cell line) cells stained with ab14734 (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 (ab14734, 1 µg/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 IgG2b [PLPV219] (**ab91366**, 2 µg/1x10^6 cells) used under the same conditions.

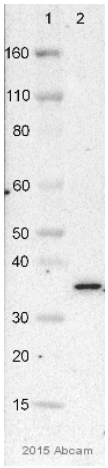
Acquisition of >5,000 events was performed.

This antibody gave a positive signal in HepG2 cells fixed with 80% methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

Immunofluoresence using ab14734 at 0.2 µg/ml on human fibroblasts (red).
Nuclei were labeled with DAPI (blue).



Western blot - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)
Image courtesy of an anonymous abreview.

Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734) at 1/500 dilution + MCF7 (Human breast adenocarcinoma cell line) cell lysate at 10 µg

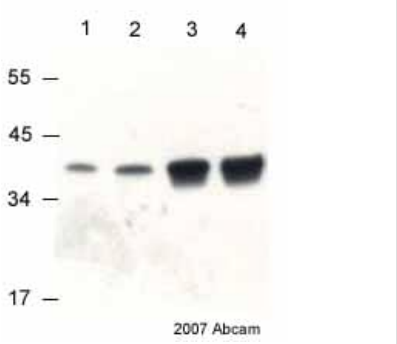
Secondary
HRP-conjugated goat anti-mouse polyclonal at 1/5000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Exposure time: 3 minutes

0.5% TBS-tween + Lait 5% NaN3 for 16 hours at 4ºC.



Western blot - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)
This image is courtesy of an anonymous Abreview

All lanes : Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734) at 1/5000 dilution

Lanes 1-2 : Rat brain cell lysate (homogenate)
Lanes 3-4 : Rat brain cell lysate (mitochondrial)

Lysates/proteins at 30 µg per lane.

Secondary
All lanes : HRP conjugated sheep anti-mouse IgG

Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 39 kDa

Exposure time: 5 minutes

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