abcam

Product datasheet

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



★★★★★ <u>52 Abreviews</u> <u>512 References</u> 画像数 12

製品の概要

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

製品の詳細 Mouse monoclonal [20B12AF2] to VDAC1/Porin + VDAC3

由来種 Mouse

アプリケーション 適用あり: WB, ICC/IF, Flow Cyt 種交差性 交差種: Mouse, Rat, Cow, Human

交差が予測される動物種: 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.

ポジティブ・コントロール WB: Isolated mitochondria from human, cow, rat and mouse heart. HepG2 cell lysate. ICC/IF:

HeLa cells. Human fibroblasts. Flow Cyt: HepG2 cells.

特記事項 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

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C. Do Not Freeze.

バッファー pH: 7.50

Preservative: 0.02% Sodium azide

Constituents: 0.36% HEPES, 0.88% Sodium chloride

精製度 IgG fraction

1

特記事項(精製) 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.

ポリ/モノ モノクローナル

クローン名 20B12AF2

アイソタイプ lgG2b

軽鎖の種類 kappa

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab14734の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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 lgG2b, is suitable for use as an isotype control with this antibody.

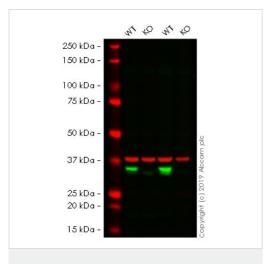
ターゲット情報

細胞内局在

 $VDAC1/Porin:\ Mitochondrion\ outer\ membrane.\ Cell\ membrane.\ VDAC3:\ Mitochondrial\ outer$

membrane.

画像



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, <u>ab181602</u> 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.

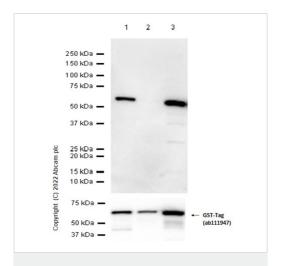
20 ls Abcam

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

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.

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

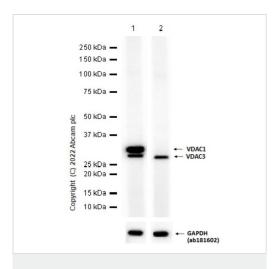
Lane 3: MEF (Mus musculus Embryo Fibroblast) whole cell lysate with NFDM/TBST

Lane 4: Rat brain tissue lysate with NFDM/TBST

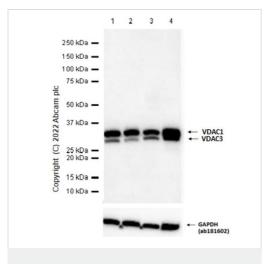
Lysates/proteins at 20 µg per lane.

Blocking peptides at 5 % per lane.

Observed band size: 33 kDa



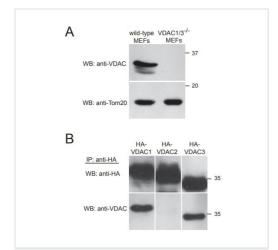
Western blot - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)



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

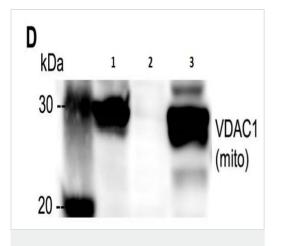
Exposure time: 5 seconds

Diluting buffer: 5% NFDM /TBST



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

Sun Yet 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.



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.

(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.

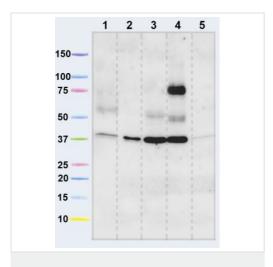
Molecular weights are indicated in kDa.

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.

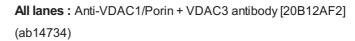
Lane 1: Whole cell lysate

Lane 2: Cytosolic fraction

Lane 3: Mitochondrial-enriched fraction



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



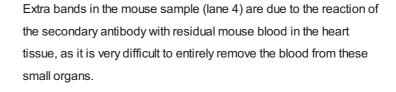
Lane 1: Isolated mitochondria from human heart at 15 μg
Lane 2: Isolated mitochondria from bovine heart at 6 μg
Lane 3: Isolated mitochondria from rat heart at 30 μg

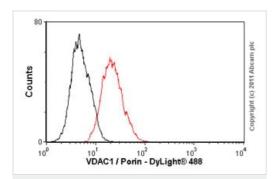
Lane 4: Isolated mitochondria from mouse heart at 30 µg

Lane 5 : HepG2 (Human liver hepatocellular carcinoma cell line)

cell lysate at 30 µg

Observed band size: 37 kDa





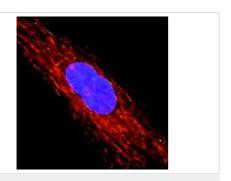
Flow Cytometry - Anti-VDAC1/Porin + VDAC3 antibody [20B12AF2] (ab14734)

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 μ g/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight[®] 488 goat anti-mouse lgG (H+L) (<u>ab96879</u>) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse lgG2b [PLPV219] (<u>ab91366</u>, 2 μ g/1x10⁶ 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 μ g/ml on human fibroblasts (red).

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

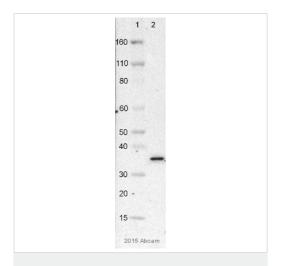
1/500 dilution + MCF7 (Human breast adenocarcinoma cell line)

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

Nuclei were labeled with DAPI (blue).

cell lysate at 10 µg

Secondary



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

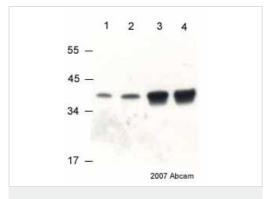
Image courtesy of an anonymous abreview.

Developed using the ECL technique.

Performed under reducing conditions.

Exposure time: 3 minutes

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



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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