abcam

Product datasheet

Anti-LAMP2A antibody - Lysosome Marker ab18528

★★★★★ 15 Abreviews 154 References 画像数 6

製品の概要

製品名 Anti-LAMP2A antibody - Lysosome Marker

製品の詳細 Rabbit polyclonal to LAMP2A - Lysosome Marker

由来種 Rabbit

特異性 Replenishment batches of our polyclonal antibody, ab18528 are tested in WB. Previous batches

were additionally validated in ICC/IF and IHC-P. These applications are still expected to work and

are covered by our Abpromise guarantee. You may also be interested in our alternative

recombinant antibody, ab125068.

アプリケーション 適用あり: WB, ICC/IF, IHC-P

種交差性 交差種: Mouse, Human

交差が予測される動物種: Dog, Monkey, Chinese hamster

免疫原 Synthetic peptide corresponding to Human LAMP2A aa 350 to the C-terminus (C terminal)

conjugated to keyhole limpet haemocyanin. LAMP2 has 3 distinct isoforms, LAMP2A, 2B & 2C.

Database link: P13473

(Peptide available as ab23322)

ポジティブ・コントロール WB: Human liver tissue lysate. ICC/IF: CaCo2 cells, HeLa, MCF7 and HepG2 cells. IHC-P:

Human breast adenocarcinoma tissue.

特記事項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 short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

80°C. Avoid freeze / thaw cycle.

バッファー pH: 7.40

Preservative: 0.02% Sodium azide

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Constituent: PBS

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising agent. If you would like information about the formulation of a specific lot, please contact our scientific support team who will be happy to help.

精製度 Immunogen affinity purified

ポリ/モノ ポリクローナル

アイソタイプ lgG

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ab18528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	★★★★☆ (8)	Use a concentration of 0.5 - 1 µg/ml. Detects a band of approximately 105 kDa (predicted molecular weight: 100 kDa). Abcam recommends using 3% milk as the blocking agent.
ICC/IF	★★★★ <u>(4)</u>	Use a concentration of 0.04 - 1 µg/ml.
IHC-P	**** <u>(1)</u>	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

ターゲット情報

機能 Implicated in tumor cell metastasis. May function in protection of the lysosomal membrane from autodigestion, maintenance of the acidic environment of the lysosome, adhesion when expressed on the cell surface (plasma membrane), and inter- and intracellular signal transduction. Protects cells from the toxic effects of methylating mutagens.

組織特異性 Isoform LAMP-2A is highly expressed in placenta, lung and liver, less in kidney and pancreas, low in brain and skeletal muscle. Isoform LAMP-2B is highly expressed in skeletal muscle, less in brain, placenta, lung, kidney and pancreas, very low in liver.

関連疾患 Danon disease

配列類似性 Belongs to the LAMP family.

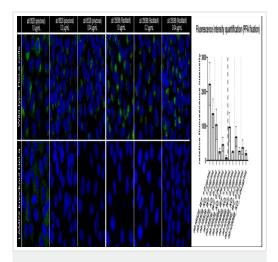
翻訳後修飾 O- and N-glycosylated; some of the 16 N-linked glycans are polylactosaminoglycans.

細胞内局在 Cell membrane. Endosome membrane. Lysosome membrane. This protein shuttles between

lysosomes, endosomes, and the plasma membrane.

製品の状態 Alternative splicing produces 3 isoforms.

画像

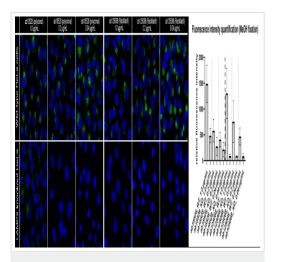


Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody - Lysosome Marker (ab18528)

Side-by-side comparison of ICC performance using the rabbit polyclonal ab18528 and RabMab® <u>ab125068</u>. Staining was performed on wild-type HeLa cells (top panel) and LAMP2 knockout HeLa cells (bottom panel, available as <u>ab255402</u>). The cells were fixed with 4% PFA (10 min), permeabilized with 0.1% Tween-20 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 ab18528 or <u>ab125068</u> overnight at +4°C at 3 different concentrations: 1.0 μ g/mL, 0.2 μ g/mL and 0.04 μ g/mL. Secondary antibody incubation was at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (<u>ab150081</u>) (shown in green) at 1/1000 and nuclear DNA was labelled with DAPI (shown in blue).

Images were acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Some cytoplasmic cross-reactivity is seen using ab18528 at 1.0 μ g/mL, but further titration of the antibody improves the ICC staining result. The RabMab® <u>ab125068</u> shows negligible non-specific staining across the dilution range. Quantification of the antibody signal was performed using a minimum of 135 cells and data are presented as mean \pm SD.

Optimal dilutions/concentrations may vary across different cell types/experiment conditions and should be determined by the end user.



Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody - Lysosome Marker (ab18528)

Side-by-side comparison of ICC performance using the rabbit polyclonal ab18528 and RabMab ab125068. Staining was performed on wild-type HeLa cells (top panel) and LAMP2 knockout HeLa cells (bottom panel, available as ab255402). The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Tween-20 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 ab18528 or ab125068 overnight at +4°C at 3 different concentrations: 1.0 μ g/mL, 0.2 μ g/mL and 0.04 μ g/mL. Secondary antibody incubation was at room temperature for 1h with a goat secondary antibody to Rabbit lgG (Alexa Fluor 488) (ab150081) (shown in green) at 1/1000 and nuclear DNA was labelled with DAPI (shown in blue).

Images were acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Some cytoplasmic cross-reactivity is seen using

ab18528 at 1.0 μ g/mL, but further titration of the antibody improves the ICC staining result. The RabMab[®] **ab125068** shows negligible non-specific staining across the dilution range. Quantification of the antibody signal was performed using a minimum of 180 cells and data are presented as mean \pm SD.

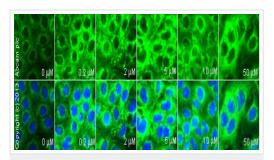
Optimal dilutions/concentrations may vary across different cell types/experiment conditions and should be determined by the end user.

ab18528 ab7291

Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody - Lysosome Marker (ab18528)

ab18528 staining LAMP2 in MCF7 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% PBS-Tween 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 overnight at 4°C with ab18528 at 1µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

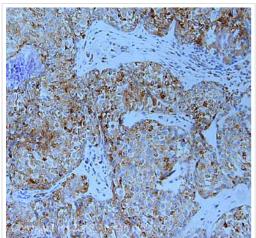
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody - Lysosome Marker (ab18528)

ab18528 staining Lamp2A in CaCO2 cells treated with SB 202190 (ab120638), by ICC/IF. Increase of Lamp2A expression correlates with increased concentration of SB 202190, as described in literature.

The cells were incubated at 37° C for 3 hours in media containing different concentrations of <u>ab120638</u> (SB 202190) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with ab18528 (5 µg/ml) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A DyLight 488 anti-rabbit polyclonal antibody (<u>ab96899</u>) at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LAMP2A antibody -Lysosome Marker (ab18528)

All lanes : Anti-LAMP2A antibody - Lysosome Marker (ab18528) at 1 µg/ml (blocked with 3% Milk)

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen

retrieval conditions, primary antibody concentration and antibody

IHC image of Lamp2A staining in human breast adenocarcinoma formalin fixed paraffin embedded tissue section, performed on a Leica Bond™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab18528, 5µg/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

Lane 1: MEF1 (Mouse embryonic fibroblast cell line) Whole Cell

Lane 2: Lung (Mouse) Tissue Lysate

Lane 3: Human liver tissue lysate - total protein (ab29889)

Lane 4: Human liver left lobe tissue lysate - membrane extract

(ab29086)

incubation times.

Lysates/proteins at 20 µg per lane.

25D-15D-1DD-75-Copyrght 82014 Abcam plc 25

Western blot - Anti-LAMP2A antibody - Lysosome Marker (ab18528)

Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 100 kDa Observed band size: 105 kDa

Additional bands at: 30 kDa, 35 kDa, 55 kDa. We are unsure as

to the identity of these extra bands.

Exposure time: 8 minutes

Abcam recommends using 3% milk as the blocking agent.

Lanes 3 and 4 are human liver tissue lysates, total protein.

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