abcam

Product datasheet

Anti-LAMP2A antibody [EPR4207(2)] - BSA and Azide free ab240018



リコンピナント

RabMAb

画像数 15

製品の概要

製品名 Anti-LAMP2A antibody [EPR4207(2)] - BSA and Azide free

製品の詳細 Rabbit monoclonal [EPR4207(2)] to LAMP2A - BSA and Azide free

由来種 Rabbit

特異性 LAMP2A is highly expressed in placenta, lung and liver, less in kidney and pancreas, low in brain

and skeletal muscle (PMID: 10212251PubMed:7488019, PubMed:26856698).

For better using it in tissue with low expression level, we suggest optimizing experimental protocols (increasing lysate amount, using lower dilution or higher sensitivity ECL substrate).

アプリケーション 適用あり: IHC-P, ICC/IF, Flow Cyt (Intra), IP, WB

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール IHC-P: Human placenta and liver tissue. Mouse and rat liver tissue. IP: HeLa and RAW 264.7

whole cell lysates. Flow Cyt (intra): HeLa cells. ICC/IF: Wild-type HeLa cells.

特記事項 ab240018 is the carrier-free version of ab125068.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar[®] Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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**.

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: 7.2

Constituent: PBS

キャリア・フリー はい

精製度 Protein A purified

ポリ/モノ モノクローナル **クローン名** EPR4207(2)

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ab240018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
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
ICC/IF		Use at an assay dependent concentration. We recommend permeabilisation with 0.1% Tween-20, 5 min.
Flow Cyt (Intra)		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 120 kDa (predicted molecular weight: 45 kDa).

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.

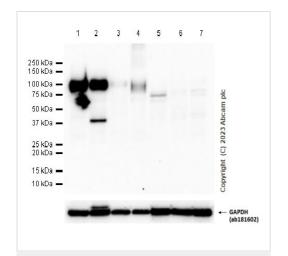
翻訳後修飾 O- and N-glycosylated; some of the 16 N-linked glycans are polylactosaminoglycans.

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



Western blot - Anti-LAMP2A antibody [EPR4207(2)] - BSA and Azide free (ab240018)

All lanes : Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) at 1/1000 dilution

Lane 1: Mouse kidney tissue lysate

Lane 2: Mouse liver tissue lysate

Lane 3: Mouse spleen tissue lysate

Lane 4: Mouse lung tissue lysate

Lane 5: Mouse brain tissue lysate

Lane 6: Mouse cerebral cortex tissue lysate

Lane 7: Mouse heart tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes: Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000

dilution

Predicted band size: 45 kDa **Observed band size:** 100 kDa

Exposure time: 40 seconds

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab125068</u>).

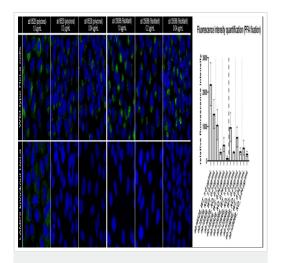
Blocking and diluting buffer and concentration: 5% NFDM /TBST.

ab181602 was used as a GAPDH loading control.

LAMP2A is highly expressed in placenta, lung and liver, less in kidney and pancreas, low in brain and skeletal muscle (PMID:

10212251PubMed:7488019, PubMed:26856698).

For better using it in tissue with low expression level, we suggest optimizing experimental protocols (increasing lysate amount, using lower dilution or higher sensitivity ECL substrate).



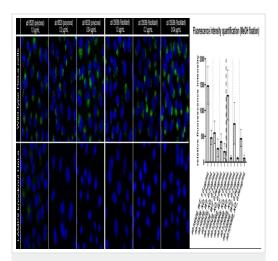
Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody [EPR4207(2)] - BSA and Azide free (ab240018)

Side-by-side comparison of ICC performance using the rabbit polyclonal <u>ab18528</u> 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 <u>ab18528</u> 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 lgG (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 $\underline{ab18528}$ at 1.0 $\mu g/mL$, but further titration of the antibody improves the ICC staining result. The RabMab® $\underline{ab125068}$ 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab125068</u>).



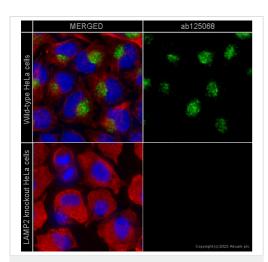
Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody [EPR4207(2)] - BSA and Azide free (ab240018)

Side-by-side comparison of ICC performance using the rabbit polyclonal <u>ab18528</u> 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 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 <u>ab18528</u> 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 lgG (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 $\underline{ab18528}$ at 1.0 $\mu g/mL$, but further titration of the antibody improves the ICC staining result. The RabMab® $\underline{ab125068}$ 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab125068</u>).



Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody [EPR4207(2)] - BSA and Azide free (ab240018)

ab125068 staining LAMP2a in 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 ab125068 at 0.04 μg/mL and ab7291 at 1 μg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit lgG (Alexa Fluor[®] 488) (ab150081) (shown in green) and goat secondary antibody to Mouse lgG (Alexa Fluor[®] 594) (ab150120) (shown in red) both at 1/1000. Nuclear DNA was labelled with DAPI (shown in blue).

In our hands, permeabilization with 0.1% Triton X-100 (5 min) resulted in greatly reduced signal and we recommend using 0.1% Tween-20 (5 min) for detecting this target.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab125068**).

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Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody [EPR4207(2)] - BSA and Azide free (ab240018)

<u>ab125068</u> staining LAMP2a in wild-type HeLa cells (top panel) and LAMP2 knockout HeLa cells (bottom panel, available as <u>ab255402</u>). The cells were fixed with 4% formaldehyde (10min), 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 <u>ab125068</u> at 0.04 μg/mL and <u>ab7291</u> at 1 μg/mL overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit lgG (Alexa Fluor[®] 488) (<u>ab150081</u>) (shown in green) and goat secondary antibody to Mouse lgG (Alexa Fluor[®] 594) (<u>ab150120</u>) (shown in red) both at 1/1000. Nuclear DNA was labelled with DAPI (shown in blue).

In our hands, permeabilization with 0.1% Triton X-100 (5 min) resulted in greatly reduced signal and we recommend using 0.1% Tween-20 (5 min) for detecting this target.

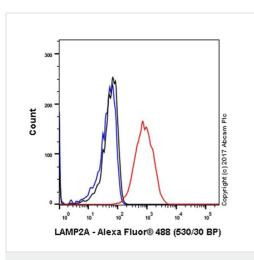
Image was acquired with a high-content analyser (Operetta CLS,

Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

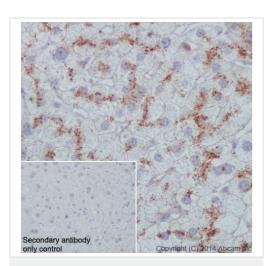
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab125068).

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling LAMP2A (red) with <u>ab125068</u> at a 1/1000 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit lgG (Alexa Fluor[®] 488) (<u>ab150077</u>) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal lgG (<u>ab172730</u>). Blue (unlabeled control) - Cells without incubation with the primary and secondary antibodies.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab125068).



Flow Cytometry (Intracellular) - Anti-LAMP2A antibody [EPR4207(2)] - BSA and Azide free (ab240018)

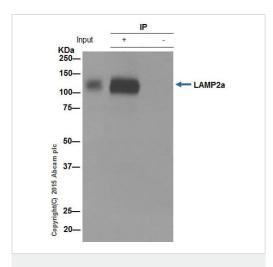


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LAMP2A antibody

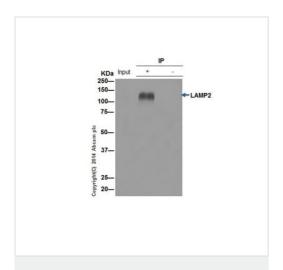
[EPR4207(2)] - BSA and Azide free (ab240018)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling LAMP2A with purified ab125068 at 1/200. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. ab97051, a HRP-conjugated goat anti-rabbit lgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab125068).



Immunoprecipitation - Anti-LAMP2A antibody
[EPR4207(2)] - BSA and Azide free (ab240018)



Immunoprecipitation - Anti-LAMP2A antibody
[EPR4207(2)] - BSA and Azide free (ab240018)

<u>ab125068</u> (purified) at 1/60 immunoprecipitating LAMP2A in HeLa whole cell lysate.

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

Lane 2 (+): ab125068 + HeLa whole cell lysate (10µg).

Lane 3 (-): Rabbit monoclonal lgG (<u>ab172730</u>) instead of <u>ab125068</u> in HeLa whole cell lysate.

For western blotting, VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1500 dilution.

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab125068).

LAMP2A was immunoprecipitated from 1mg of RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate with <u>ab125068</u> at 1/100 dilution. Western blot was performed from the immunoprecipitate using unpurified <u>ab125068</u> at 1/2000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG was used as secondary antibody at 1/1500 dilution.

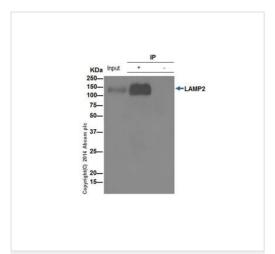
Lane 1: RAW 264.7 whole cell lysate 10ug (Input).

Lane 2: ab125068 IP in RAW 264.7 whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G (\underline{ab172730})$ instead of $\underline{ab125068}$ in RAW 264.7 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab125068</u>).



Immunoprecipitation - Anti-LAMP2A antibody
[EPR4207(2)] - BSA and Azide free (ab240018)

LAMP2A was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with unpurified <u>ab125068</u> at 1/100 dilution. Western blot was performed from the immunoprecipitate using <u>ab125068</u> at 1/2000 dilution. Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG was used as secondary antibody at 1/1500 dilution.

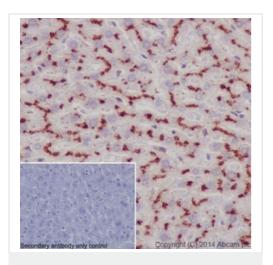
Lane 1: HeLa whole cell lysate 10ug (Input).

Lane 2: <u>ab125068</u> IP in HeLa whole cell lysate.

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of $\underline{ab125068}$ in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab125068).



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

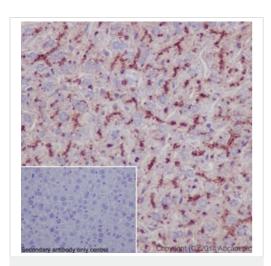
[EPR4207(2)] - BSA and Azide free (ab240018)

Immunohistochemical analysis of paraffin-embedded Rat liver labeling LAMP2A with unpurified <u>ab125068</u> at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Cytoplasmic and membrane staining on rat liver tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab125068).

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



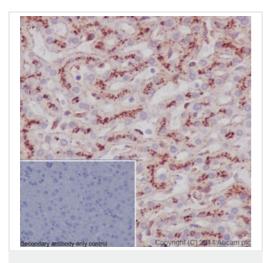
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-LAMP2A antibody
[EPR4207(2)] - BSA and Azide free (ab240018)

Immunohistochemical analysis of paraffin-embedded Mouse liver labeling LAMP2A with unpurified <u>ab125068</u> at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Cytoplasmic and membrane staining on mouse liver tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab125068).

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



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

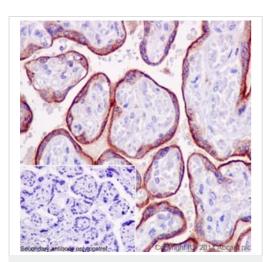
[EPR4207(2)] - BSA and Azide free (ab240018)

Immunohistochemical analysis of paraffin-embedded Human liver labeling LAMP2A with unpurified <u>ab125068</u> at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/500 dilution. Cytoplasmic and membrane staining on human liver tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab125068).

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



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

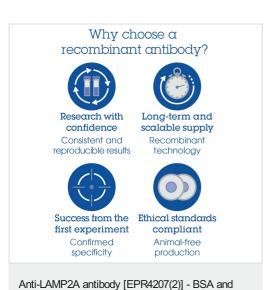
[EPR4207(2)] - BSA and Azide free (ab240018)

Immunohistochemical analysis of paraffin-embedded Human placenta labeling LAMP2A with unpurified ab125068 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic and membrane staining on human placenta tissue is observed. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/500 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab125068).

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



Azide free (ab240018)

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