abcam

ab125068

Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker

Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) is a rabbit monoclonal antibody detecting LAMP2A in **Western Blot, Flow Cytometry (Intra), IP, IHC-P, ICC/IF**. Suitable for **Human, Mouse, Rat**.

- Biophysical QC for unrivalled batch-batch consistency
- Over 50 publications

Lab Essentials | Recombinant | RabMAb | KO Validated | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR4207(2)
Purification technique	Affinity purification Protein A
Specificity	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).
Concentration	1.032 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Mouse
Dilution info	1/200 - 1/500
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/200 - 1/500
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Species	Human
Dilution info	1/200 - 1/500
Notes	Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

IP

Tested

Species	Mouse
Dilution info	1/60 - 1/100
Notes	-

Species	Human
Dilution info	1/60 - 1/100
Notes	-

Expected

Species	Rat

Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/2000
Notes	-

Species	Rat
Dilution info	1/2000
Notes	-

Species	Human
Dilution info	1/2000
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/1000
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	0.04-13 µg/mL
Notes	We recommend permeabilisation with 0.1% Tween-20, 5 min.

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information LAMP2

Function

Lysosomal membrane glycoprotein which plays an important role in lysosome biogenesis, lysosomal pH regulation and autophagy (PubMed:11082038, PubMed:18644871, PubMed:24880125, PubMed:27628032, PubMed:36586411, PubMed:37390818, PubMed:8662539). Acts as an important regulator of lysosomal lumen pH regulation by acting as a direct inhibitor of the proton channel TMEM175, facilitating lysosomal acidification for optimal hydrolase activity (PubMed:37390818). Plays an important role in chaperone-mediated autophagy, a process that mediates lysosomal degradation of proteins in response to various stresses and as part of the normal turnover of proteins with a long biological half-live (PubMed:11082038, PubMed:18644871, PubMed:24880125, PubMed:27628032, PubMed:36586411, PubMed:8662539). Functions by binding target proteins, such as GAPDH, GPX4, NLRP3 and MLLT11, and targeting them for lysosomal degradation (PubMed:11082038, PubMed:18644871, PubMed:24880125, PubMed:36586411, PubMed:8662539). In the chaperone-mediated autophagy, acts downstream of chaperones, such as HSPA8/HSC70, which recognize and bind substrate proteins and mediate their recruitment to lysosomes, where target proteins bind LAMP2 (PubMed:36586411). Plays a role in lysosomal protein degradation in response to starvation (By similarity). Required for the fusion of autophagosomes with lysosomes during autophagy (PubMed:27628032). Cells that lack LAMP2 express normal levels of VAMP8, but fail to accumulate STX17 on autophagosomes, which is the most likely explanation for the lack of fusion between autophagosomes and lysosomes (PubMed:27628032). Required for normal degradation of the contents of autophagosomes (PubMed:27628032). Required for efficient MHC class II-mediated presentation of exogenous antigens via its function in lysosomal protein degradation; antigenic peptides generated by proteases in the endosomal/lysosomal compartment are captured by nascent MHC II subunits (PubMed:15894275, PubMed:20518820). Is not required for efficient MHC class II-mediated presentation of endogenous antigens (PubMed:20518820). Isoform LAMP-2C. Modulates chaperone-mediated autophagy. Decreases presentation of endogenous antigens by MHCII. Does not play a role in the presentation of exogenous and membrane-derived antigens by MHCII. (Microbial infection) Supports the FURIN-mediated cleavage of mumps virus fusion protein F

by interacting with both FURIN and the unprocessed form but not the processed form of the viral protein F.

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Stable for 12 months at -20°C

Notes

Product Specifications

Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P, IP, WB in human, mouse, rat samples.
Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) specifically detects LAMP2A (UniProt ID: P13473; Molecular weight: 42kDa) and is sold in 100 µL and 1 mL selling sizes.

Quality and Validation

Abcam's high quality manufacturing and validation processes ensure Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.
The specificity of Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) has been confirmed by testing in knockout samples.
Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) has been cited over 53 times in peer reviewed journals and is trusted by the scientific community.

Related Products
Conjugation-ready, carrier free format available for antibody clone EPR4207(2) - ab240018.
Antibody clone EPR4207(2) is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 594, Alexa Fluor® 555, Alexa Fluor® 647, PE, APC, HRP, Alkaline Phosphatase, Alexa Fluor® 488, Alexa Fluor® 568, Alexa Fluor® 750 (ab281931, ab282009, ab284133, ab305387, ab305388, ab305389, ab309054, ab309845, ab312675, ab320999).
Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	LAMP2A also known as lysosome-associated membrane protein 2A functions as a lysosomal marker and plays a role in the lysosome size and structure regulation. Its molecular weight is approximately 45 kDa. This protein is expressed mainly in tissues with high metabolic activity such as liver kidney and heart. LAMP2A is an integral component of the lysosomal membrane and is important for the proper function and maintenance of lysosomes which are cellular organelles involved in the degradation and recycling of macromolecules.
Biological function summary	LAMP2A facilitates chaperone-mediated autophagy (CMA) a specific mechanism for the selective degradation of proteins. It is not part of a larger complex but rather acts independently within the lysosomal membrane. This transport functions through recognizing cytosolic proteins containing a KFERQ-like motif which are then translocated into the lysosome for degradation. LAMP2A upholds cellular homeostasis and is important in adapting to cellular stress by facilitating the turnover of damaged or unnecessary proteins.
Pathways	LAMP2A is intimately involved in the CMA pathway. This pathway is important for regulating protein quality control often linked with other autophagic processes. LAMP2A also interacts with the heat shock cognate protein (HSC70) which assists in unfolding and transporting substrate proteins to lysosomes. Through these interactions LAMP2A helps maintain cellular proteostasis which is essential for cellular function and survival especially during stress conditions.
Associated diseases and disorders	LAMP2A is connected to lysosomal storage disorders such as Danon disease. This disease is characterized by the accumulation of autophagic vacuoles due to defects in lysosomal function. LAMP2A mutations alter lysosomal degradation capabilities leading to severe cardiomyopathy and myopathy. Additionally LAMP2A has implications in neurodegenerative diseases like Parkinson's disease due to its role in protein degradation pathways. In these contexts malfunction or altered expression of LAMP2A can disrupt cellular clearance processes contributing to pathogenesis along with proteins such as alpha-synuclein.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

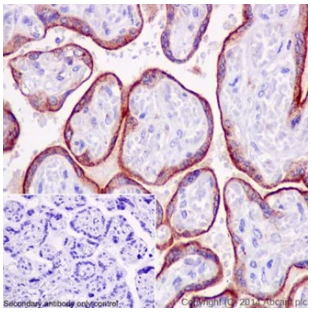
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

22 product images

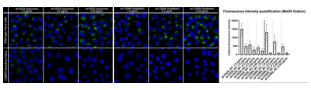


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-LAMP2A antibody

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 IgG H&L (HRP) (ab97051) at 1/500 dilution.

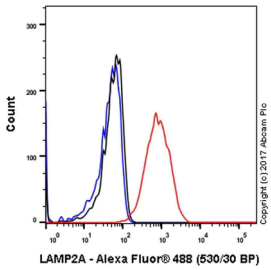
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

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 µg/mL, 0.2 µg/mL and 0.04 µg/mL.

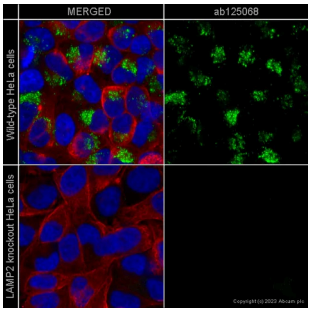
Secondary antibody incubation was at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (ab150081) (shown in green) at 1/1000 and nuclear DNA was labelled with DAPI (shown in blue). Images were acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown. Some cytoplasmic cross-reactivity is seen using ab18528 at 1.0 µg/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 ± SD.Optimal dilutions/concentrations may vary across different cell types/experiment conditions and should be determined by the end user.



Flow Cytometry (Intracellular) - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A Flow Cytometry (Intracellular) staining using rabbit Anti-LAMP2A antibody

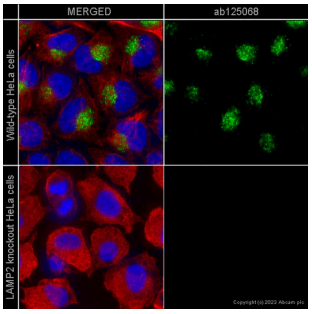
Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling LAMP2A (red) with ab125068 at a 1/1000 dilution. Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. A goat anti-rabbit IgG (Alexa Fluorr® 488) (ab150077) was used as the secondary antibody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



Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

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 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 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 IgG (Alexa Fluor® 488) (ab150081) (shown in green) and goat secondary antibody to Mouse IgG (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.

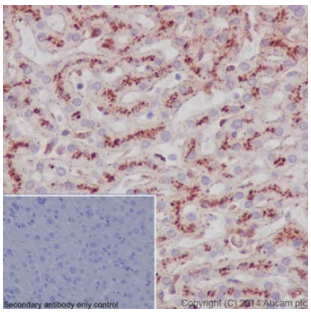
Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.



Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

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 IgG (Alexa Fluor® 488) (ab150081) (shown in green) and goat secondary antibody to Mouse IgG (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.

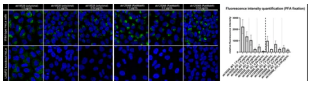


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-LAMP2A antibody

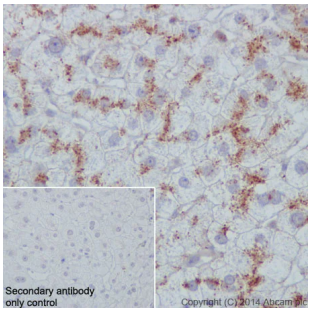
Immunohistochemical analysis of paraffin-embedded Human liver labeling LAMP2A with unpurified ab125068 at 1/500 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution. Cytoplasmic and membrane staining on human liver tissue is observed. Counter stained with Hematoxylin.
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/500 dilution.

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



Immunocytochemistry/ Immunofluorescence - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

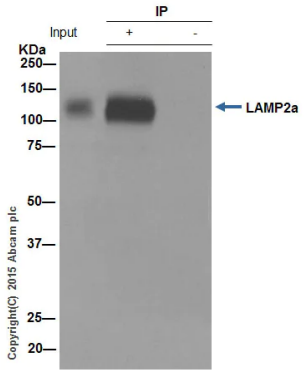
Side-by-side comparison of ICC performance using the rabbit polyclonal ab18528 and RabMab® ab125068. Staining was performed on wild-type HeLa cells (top panel) and LAMP2 knockout HeLa cells (bottom panel, available as ab255402). The cells were fixed with 4% PFA (10 min), permeabilized with 0.1% Tween-20 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 ab125068 overnight at +4°C at 3 different concentrations: 1.0 µg/mL, 0.2 µg/mL and 0.04 µg/mL. Secondary antibody incubation was at room temperature for 1h with a goat secondary antibody to Rabbit IgG (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 µg/mL, but further titration of the antibody improves the ICC staining result. The RabMab® ab125068 shows negligiblenon-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 ± SD.Optimal dilutions/concentrations may vary across different cell types/experiment conditions and should be determined by the end user.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-LAMP2A antibody

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 IgG (H+L) was used as the secondary antibody (1/500). Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.



Immunoprecipitation - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

ab125068 (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 IgG (ab172730) instead of ab125068 in HeLa whole cell lysate.

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

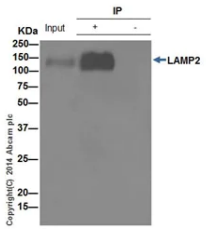
Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

All lanes:
Immunoprecipitation - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

Predicted band size: 45 kDa

Observed band size: 120 kDa



Immunoprecipitation - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A was immunoprecipitated from 1mg of HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate with unpurified ab125068 at 1/100 dilution. Western blot was performed from the immunoprecipitate using ab125068 at 1/2000 dilution. Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG was used as secondary antibody at 1/1500 dilution.
Lane 1: HeLa whole cell lysate 10ug (Input).

Lane 2: ab125068 IP in HeLa whole cell lysate.

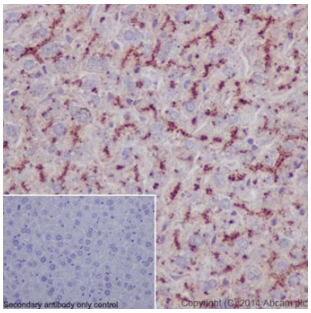
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab125068 in HeLa whole cell lysate.

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

All lanes:
Immunoprecipitation - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

Predicted band size: 45 kDa

Observed band size: 120 kDa



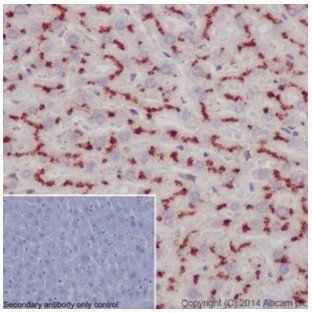
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-LAMP2A antibody

Immunohistochemical analysis of paraffin-embedded Mouse liver 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 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 IgG H&L (HRP) (ab97051) at 1/500 dilution.

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

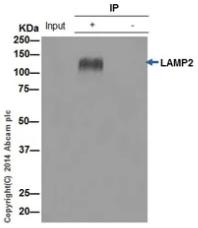


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-LAMP2A antibody

Immunohistochemical analysis of paraffin-embedded Rat liver 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 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 IgG H&L (HRP) (ab97051) at 1/500 dilution.

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



Immunoprecipitation - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

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

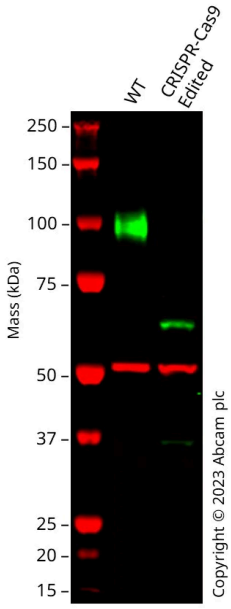
Lane 3: Rabbit monoclonal IgG (ab172730) instead of ab125068 in RAW 264.7 whole cell lysate.

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

All lanes:
Immunoprecipitation - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

Predicted band size: 45 kDa

Observed band size: 120 kDa



Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A Western blot staining using rabbit Anti-LAMP2A antibody

False colour image of Western blot: Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker staining at 1/2000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (ab7291) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab125068 was shown to bind specifically to LAMP2A. A band was observed at 100 kDa in wild-type HeLa cell lysates with no signal observed at this size in LAMP2 CRISPR-Cas9 edited cell line ab255402 (CRISPR-Cas9 edited cell lysate ab263861). The band observed in the CRISPR-Cas9 edited lysate lane below 100 kDa is likely to represent a truncated form of LAMP2A. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and LAMP2 CRISPR-Cas9 edited HeLa cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) at 1/2000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

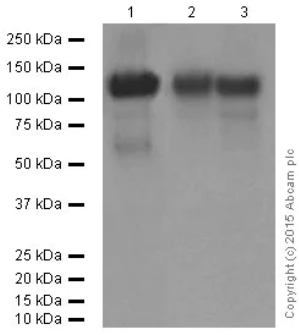
Lane 2:
LAMP2 CRISPR-Cas9 edited HeLa cell lysate at 20 µg

Secondary

All lanes:
Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 100 kDa



Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

Blocking and dilution buffer: 5% NFDM/TBST.
May be seen at ~50 kDa representing the unglycosylated isoforms of LAMP2 and ~120 kDa representing the glycosylated form.

All lanes:
Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) at 1/10000 dilution

Lane 1:
Jurkat cell lysate at 20 µg

Lane 2:
ECV-304 cell lysate at 20 µg

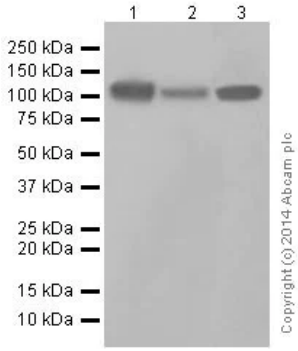
Lane 3:
JAR cell lysate at 20 µg

Secondary

All lanes:
Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 45 kDa

Observed band size: 120 kDa



Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

Blocking and Diluting buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) at 1/5000 dilution

Lane 1:
Jurkat (Human T cell leukemia cells from peripheral blood) whole cell lysate at 20 µg

Lane 2:
ECV-304 (Human urinary bladder cancer cell line) whole cell lysate at 20 µg

Lane 3:
JAR (Human placenta choriocarcinoma cell line) whole cell lysate at 20 µg

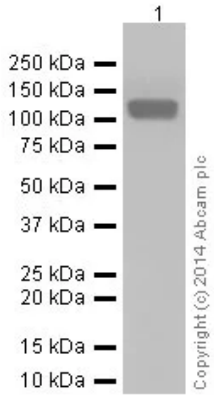
Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 37 kDa, 45 kDa

Observed band size: 120 kDa

Exposure time: 30s



Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

Blocking and Diluting buffer and concentration: 5% NFDM/TBST
All lanes:
Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) at 1/5000 dilution

All lanes:
HeLa (Human epithelial cells from cervix adenocarcinoma) whole cell lysate at 20 µg

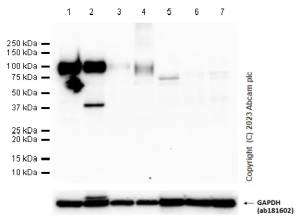
Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 45 kDa

Observed band size: 120 kDa

Exposure time: 3min



Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

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

All lanes:
Western blot at 20 µg

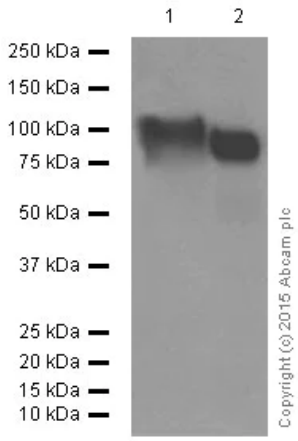
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 45 kDa

Observed band size: 100 kDa

Exposure time: 40s



Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A Western blot staining using rabbit Anti-LAMP2A antibody

Blocking and dilution buffer: 5% NFDM/TBST.
Difference in MW may be caused by different degree of glycosylation.

All lanes:
Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) at 1/2000 dilution

Lane 1:
Mouse kidney tissue lysate at 20 µg

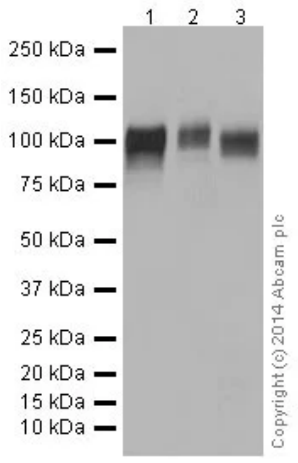
Lane 2:
Rat kidney tissue lysate at 20 µg

Secondary

All lanes:
Peroxidase-conjugated goat anti-rabbit IgG, (H+L) at 1/1000 dilution

Predicted band size: 45 kDa

Observed band size: 100 kDa



Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A Western blot staining using rabbit Anti-LAMP2A antibody

Blocking and Diluting buffer and concentration: 5% NFDM /TBST
All lanes:
Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) at 1/2000 dilution

Lane 1:
Mouse kidney at 10 µg

Lane 2:
RAW 264.7 (Mouse macrophage cells transformed with Abelson murine leukemia virus) whole cell lysate at 10 µg

Lane 3:
NIH/3T3 (Mouse embyro fibroblast cells) whole cell lysate at 10 µg

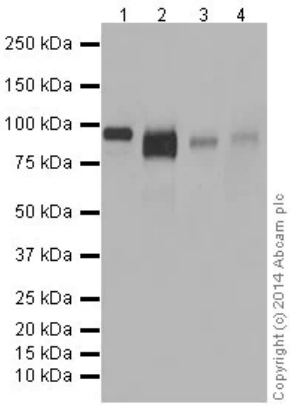
Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 45 kDa

Observed band size: 120 kDa

Exposure time: 3min



Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

Blocking and Diluting buffer and concentration: 5% NFDM/TBST.
All lanes:
Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068) at 1/2000 dilution

Lane 1:
Rat liver at 10 µg

Lane 2:
Rat kidney at 10 µg

Lane 3:
C6 (Rat glial tumor cells) whole cell lysate at 10 µg

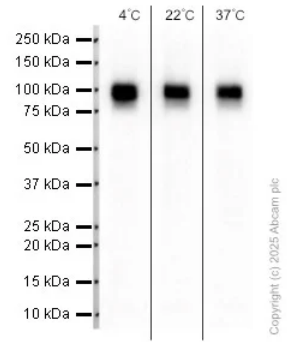
Lane 4:
PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysate at 10 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 45 kDa

Exposure time: 3min



Western blot - Anti-LAMP2A antibody [EPR4207(2)] - Lysosome Marker (ab125068)

LAMP2A Western blot staining of Mouse kidney lysate using rabbit Anti-LAMP2A antibody

This blot was produced using 4-20% SDS-PAGE containing 15 µg of mouse kidney lysate per lane at 150V for 1hr before being transferred onto a 0.45 µm PVDF membrane at 75V for 1hr. The membrane was then blocked for 1hr using 5% NFDM/TBST, then incubated with ab125068 (1/1000) at room temperature for 1hr. After being washed three times in TBST, the membrane was incubated with Peroxidase conjugated goat anti-rabbit IgG (H+L) (ab97051) at 1/20,000 dilution for 1hr at room temperature. The membrane was washed three times again. Then the signal was developed using the ECL technique.

ab125068 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in WB. The image shows the band intensity remains relatively constant for temperatures ranging between 4°C, 22°C and 37°C, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam’ s transition from cold⊠chain to ambient shipping.

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

All lanes:
Mouse kidney lysate at 15 µg with NDFM/TBST

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Exposure time: 40s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.