abcam

Product datasheet

Anti-LAMP2 antibody [H4B4] - Lysosome Marker ab25631

19 Abreviews 175 References 7 Images

Overview

Product name	Anti-LAMP2 antibody [H4B4] - Lysosome Marker
Description	Mouse monoclonal [H4B4] to LAMP2 - Lysosome Marker
Host species	Mouse
Tested applications	**Suitable for:** ICC, ICC/IF, IHC-Fr, WB, IHC-FoFr, Flow Cyt (Intra), IHC-P
Species reactivity	**Reacts with:** Human, Rhesus monkey, African green monkey **Does not react with:** Mouse, Rat
Immunogen	Tissue, cells or virus corresponding to Human LAMP2. Human adherent peripheral blood cells
Positive control	Flow Cyt (Intra): THP1 cells.
General notes	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

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 8.20 Constituent: 100% Borate buffered saline
Purity	Affinity purified
Clonality	Monoclonal
Clone number	H4B4
Isotype	IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab25631 in the following tested applications.

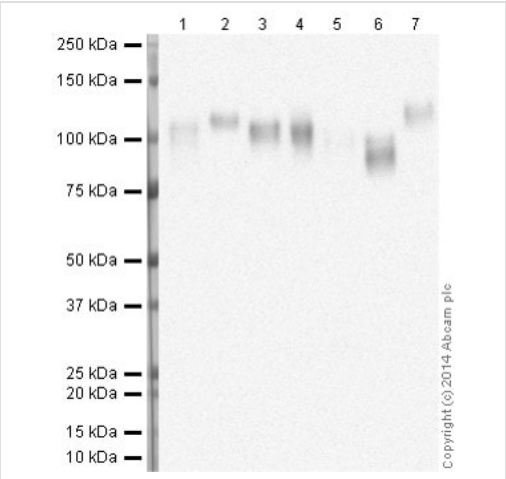
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC	★★★★☆ (1)	Use at an assay dependent concentration.
ICC/IF	★★★★★ (11)	Use at an assay dependent concentration.
IHC-Fr	★★★★☆ (1)	Use at an assay dependent concentration.
WB	★★★★★ (5)	Use at an assay dependent concentration. In our hands milk blocking is gives superior results to BSA blocking for this product.
IHC-FoFr		Use at an assay dependent concentration. (PMID 19837699).
Flow Cyt (Intra)		Use 0.5-1µg for 10^6 cells. **ab170190** - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★★ (1)	Use a concentration of 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Target

Function	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.
Tissue specificity	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.
Involvement in disease	Defects in LAMP2 are the cause of Danon disease (DAND) [MIM:300257]; also known as glycogen storage disease type 2B (GSD2B). DAND is a lysosomal glycogen storage disease characterized by the clinical triad of cardiomyopathy, vacuolar myopathy and mental retardation. It is often associated with an accumulation of glycogen in muscle and lysosomes.
Sequence similarities	Belongs to the LAMP family.
Post-translational modifications	O- and N-glycosylated; some of the 16 N-linked glycans are polylactosaminoglycans.
Cellular localization	Cell membrane. Endosome membrane. Lysosome membrane. This protein shuttles between lysosomes, endosomes, and the plasma membrane.
Form	Alternative splicing produces 3 isoforms.

Images



Western blot - Anti-LAMP2 antibody [H4B4] - Lysosome Marker (ab25631)

All lanes : Anti-LAMP2 antibody [H4B4] - Lysosome Marker (ab25631) at 1/500 dilution

Lane 1 : HeLa cell lysate
Lane 2 : Jurkat cell lysate
Lane 3 : Human liver lysate
Lane 4 : Human liver membrane fraction lysate
Lane 5 : Human skeletal muscle lysate
Lane 6 : Human brain lysate
Lane 7 : Human kidney lysate

Lysates/proteins at 20 µg per lane.

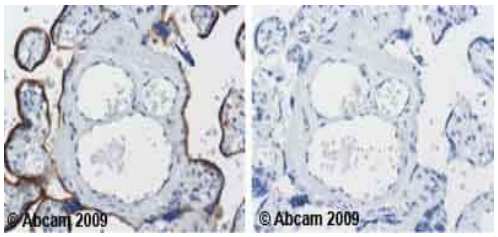
Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/50000 dilution

Performed under reducing conditions.

Additional bands at: 100 kDa (possible post-translational modification), 110 kDa (possible post-translational modification), 120 kDa (possible post-translational modification)

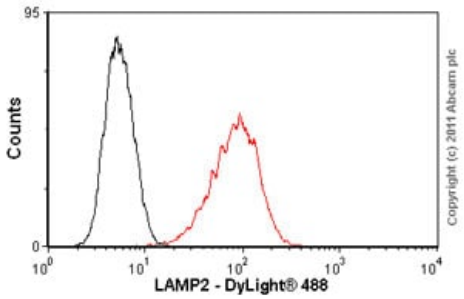
Exposure time: 20 minutes

This blot was produced using a 4-12% Bis-Tris gel under the MOPS buffer system under denaturing, reducing conditions. The gel was run at 200V for 60 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes. After transfer, the membrane was blocked for an hour in 3% milk before being incubated overnight at 4°C with mouse monoclonal [H4B4] to LAMP2 (ab25631; diluted 1:5000). Antibody binding was detected using peroxidase labelled goat anti-mouse IgG (**ab97040**; diluted 1:50000) for an hour at room temperature and visualised using ECL development solution.



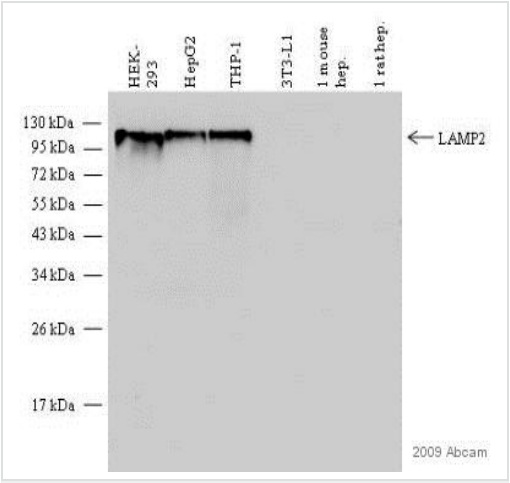
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAMP2 antibody [H4B4] - Lysosome Marker (ab25631)

Ab25631 staining human normal placenta. Staining is localized to the cytoplasm.
Left panel: with primary antibody at 1 ug/ml. Right panel: isotype control.
Sections were stained using an automated system DAKO Autostainer Plus , at room temperature. Sections were rehydrated and antigen retrieved with the Dako 3-in-1 AR buffers EDTA pH 9.0 in a DAKO PT Link. Slides were peroxidase blocked in 3% H2O2 in methanol for 10 minutes. They were then blocked with Dako Protein block for 10 minutes (containing casein 0.25% in PBS) then incubated with primary antibody for 20 minutes and detected with Dako Envision Flex amplification kit for 30 minutes. Colorimetric detection was completed with Diaminobenzidine for 5 minutes. Slides were counterstained with Haematoxylin and coverslipped under DePeX. Please note that for manual staining we recommend to optimize the primary antibody concentration and incubation time (overnight incubation), and amplification may be required.



Flow Cytometry (Intracellular) - Anti-LAMP2 antibody [H4B4] - Lysosome Marker (ab25631)

Overlay histogram showing THP1 cells stained with ab25631 (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 (ab25631, 0.5µg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (**ab96879**) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] (**ab91353**, 2µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in THP1 cells fixed with methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



Western blot - Anti-LAMP2 antibody [H4B4] - Lysosome Marker (ab25631)

This image is of an Abreciew submitted by Daniel Rodriguez.

All lanes : Anti-LAMP2 antibody [H4B4] - Lysosome Marker (ab25631) at 1/500 dilution

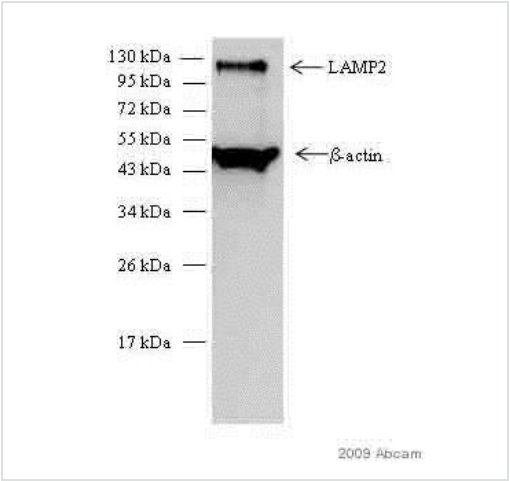
Lane 1 : HEK293 cell lysate at 30 µg
Lane 2 : HepG2 at 30 µg
Lane 3 : THP-1 at 30 µg
Lane 4 : 3T3-L1 at 30 µl
Lane 5 : Mouse hepatocytes at 30 µl
Lane 6 : Rat hepatocytes at 30 µl

Secondary
All lanes : HRP conjugated Goat anti-Mouse IgG at 1/2000 dilution

Developed using the ECL technique.

Exposure time: 30 seconds

Along with THP-1 macrophages, other human cell lines were loaded, and the 110 kDa mature band for LAMP2 was detected in all the samples. On the other hand, mouse and rat cells were negative. The antibody works really good on human samples, detecting a single 110 kDa band but it's not suitable to use for mouse or rat samples.



Western blot - Anti-LAMP2 antibody [H4B4] - Lysosome Marker (ab25631)

This image was kindly supplied by Daniel Rodriguez by Abreview

Anti-LAMP2 antibody [H4B4] - Lysosome Marker (ab25631) at 1/500 dilution + whole cell lysate prepared from THP-1 macrophages at 30 µg

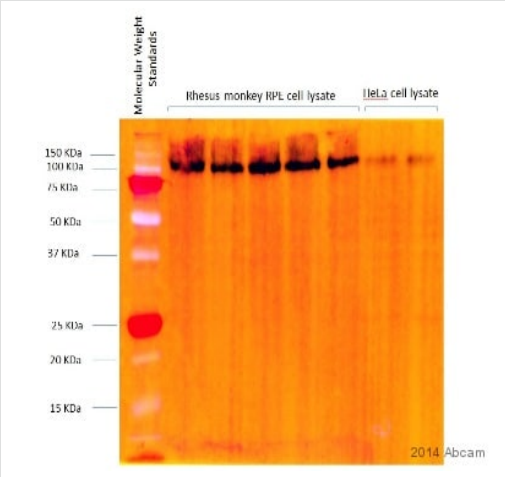
Secondary
Goat anti-mouse IgG conjugated to HRP at 1/2000 dilution

Developed using the ECL technique.

Observed band size: 110 kDa

Exposure time: 30 seconds

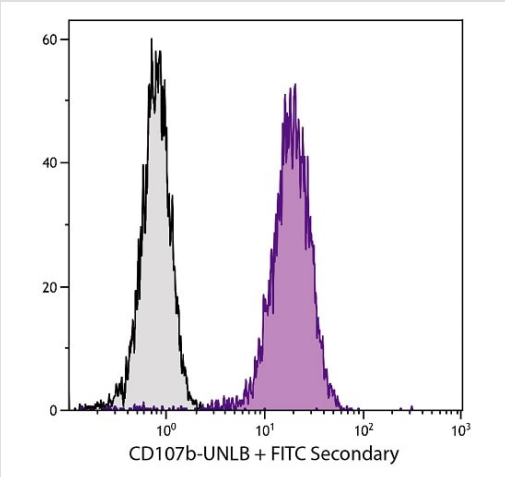
Primary antibody incubated for 12 hours at 4°C.
Gel running conditions: 12%
Blocked with 5% milk for 1 hour at 25°C.



Western blot - Anti-LAMP2 antibody [H4B4] - Lysosome Marker (ab25631)

This image is courtesy of an anonymous Abreview

Western blot analysis of Rhesus monkey primary retinal pigmented epthelium whole cell lysate and HeLa cell whole cell lysate (20µg/lane) labelling LAMP2 with ab25631 at 1/1000. An alkaline phosphatase-conjugated rabbit anti-mouse IgG was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-LAMP2 antibody [H4B4] - Lysosome Marker (ab25631)

Intracellular Flow Cytometry analysis of Human T lymphocyte cell line Jurkat labeling LAMP2 with ab25631 at 1 µg/10^6 cells dilution (purple). A Goat Anti-Mouse IgG1, Human ads-FITC was used as the secondary antibody. Grey - Isotype Control, Mouse IgG1-UNLB, followed by Goat Anti-Mouse IgG1, Human ads-FITC.

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