abcam

Product datasheet

Anti-LAMP1 antibody [H4A3] ab25630

23 Abreviews 244 References 9 Images

Overview

Product name	Anti-LAMP1 antibody [H4A3]
Description	Mouse monoclonal [H4A3] to LAMP1
Host species	Mouse
Tested applications	**Suitable for:** ICC/IF, WB, Flow Cyt (Intra), IHC-P
Species reactivity	**Reacts with:** Human
Immunogen	Tissue, cells or virus corresponding to LAMP1. Human adherent peripheral blood cells
Positive control	ICC/IF: Hela cells; Human gastric cancer cells; HaCaT keratinocytes. IHC-P: Human placenta tissue. WB: Jurkat and MCF-7 cells. Flow Cyt (Intra): Jurkat cells.
General notes	Although there are publications stating this antibody works with Mouse species (PMID 18840681), and previous batches gave positive staining on murine cells, recent batches are failing to react with this species (from customer feedback). Further feedback using this antibody with Mouse tissues would be very welcome. This product was changed from ascites to tissue culture supernatant on 3rd September 2018. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team. 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 Constituents: 0.6% Boric Acid, 0.95% Sodium borate, 0.4% Sodium chloride
Purity	Affinity purified
Clonality	Monoclonal

Clone number	H4A3
Isotype	IgG1
Light chain type	kappa

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab25630 in the following tested applications.

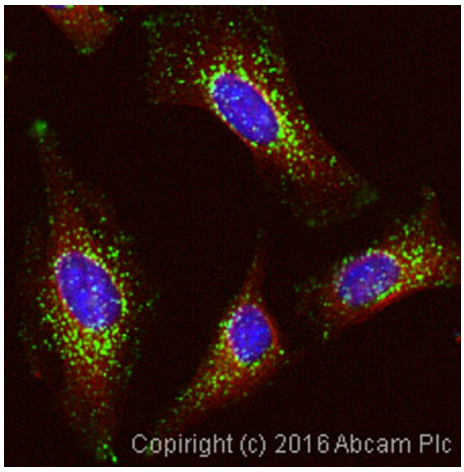
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
ICC/IF	★★★★☆ **(15)**	Use a concentration of 5 - 10 µg/ml.
WB	★★★★☆ **(6)**	1/10000. Detects a band of approximately 48 kDa (predicted molecular weight: 45 kDa).
Flow Cyt (Intra)		1/20. **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

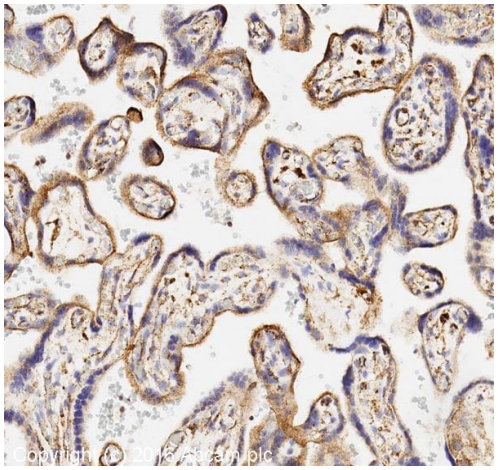
Function	Presents carbohydrate ligands to selectins. Also implicated in tumor cell metastasis.
Sequence similarities	Belongs to the LAMP family.
Post-translational modifications	O- and N-glycosylated; some of the 18 N-linked glycans are polylactosaminoglycans.
Cellular localization	Cell membrane. Endosome membrane. Lysosome membrane. This protein shuttles between lysosomes, endosomes, and the plasma membrane.

Images



Immunocytochemistry/ Immunofluorescence - Anti-LAMP1 antibody [H4A3] - Drosophila Lysosome Marker (ab25630)

Ab25630 stained Hela cells. The cells were 100% methanol fixed for 5 minutes and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Triton for 1h at room temperature to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with ab25630 at 5µg/ml and **ab202272** (Rabbit monoclonal [EP1332Y] to alpha Tubulin - Microtubule Marker (Alexa Fluor® 594) – pseudo-colored red) overnight at +4°C. The secondary antibody (pseudo-colored green) was a Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) preadsorbed (**ab150117**) used at a 1/1000 dilution for 1h at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43µM for 1hour at room temperature.

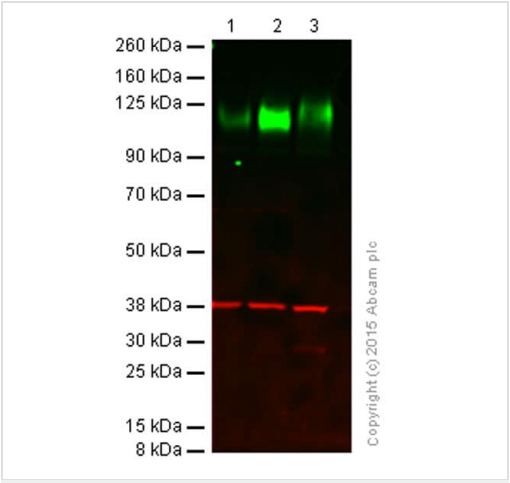


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAMP1 antibody [H4A3] - Drosophila Lysosome Marker (ab25630)

IHC image of LAMP1 staining in human placenta 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 ab25630, 1µg/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.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

**Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*



Western blot - Anti-LAMP1 antibody [H4A3] - Drosophila Lysosome Marker (ab25630)

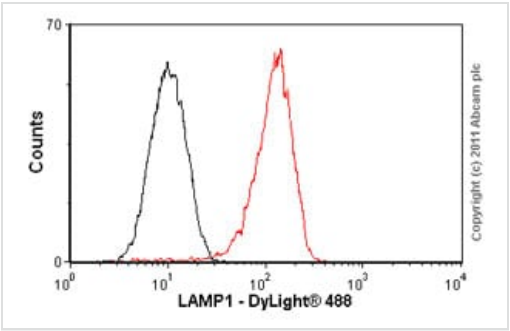
All lanes : Anti-LAMP1 antibody [H4A3] (ab25630) at 1/1000 dilution

Lane 1 : Jurkat cell lysate
Lane 2 : Jurkat membrane lysate
Lane 3 : MCF-7 cell lysate

Lysates/proteins at 20 µg per lane.

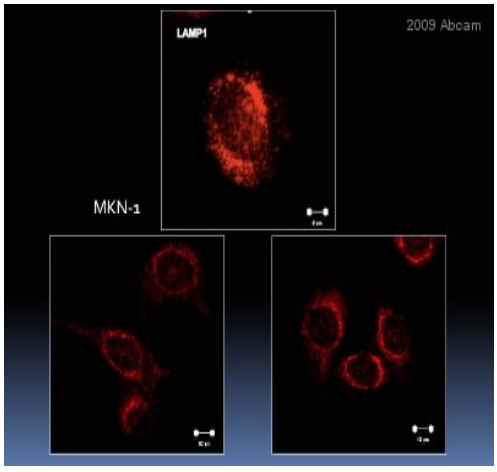
Predicted band size: 45 kDa
Observed band size: 115-120 kDa

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using Licor blocking buffer before being incubated with ab25630 (1/1000) overnight at 4°C. Antibody binding was detected using **ab9485** (rabbit anti-GAPDH); at a 1/10,000 dilution for 1hr at room temperature and then imaged using the Licor Odyssey CLx.



Flow Cytometry (Intracellular) - Anti-LAMP1 antibody [H4A3] (ab25630)

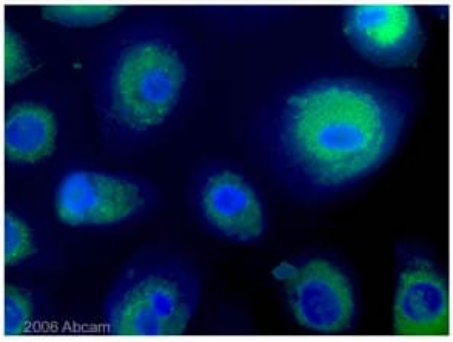
Overlay histogram showing Jurkat cells stained with ab25630 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab25630, 1/20 dilution) 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 Jurkat cells fixed with methanol (5 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-LAMP1 antibody [H4A3] - Drosophila Lysosome Marker (ab25630)

This image is courtesy of an anonymous Abreview

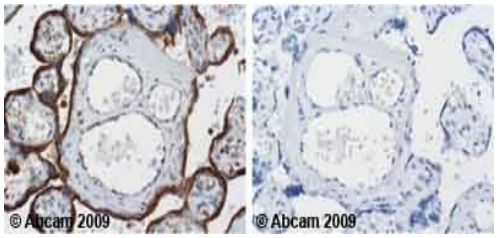
ab25630 at 1/500 dilution staining LAMP1 in human gastric cancer cells by immunocytochemistry/ immunofluorescence. Sections were methanol fixed, permeabilized in 0.5% Triton X-100 prior to blocking in 10% NGS/1% BSA for 1 hour at 25°C and then incubated with ab25630 for 2 hours at 25°C. Alexa fluor® 594 mouse polyclonal to mouse Ig, diluted 1/600, was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-LAMP1 antibody [H4A3] - Drosophila Lysosome Marker (ab25630)

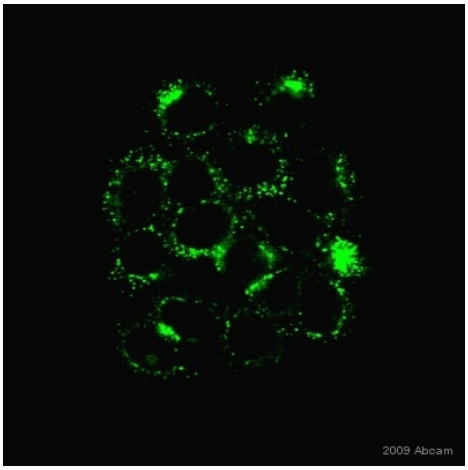
This image is courtesy of an anonymous Abreview

ab25630 at 1/100 staining human Primary Gingival epithelial cells by ICC/IF. The cells were ixed with 4% paraformaldehyde, permeabilized with 0.5% saponin and then blocked overnight with 10% goat serum, 5% BSA. The cells were incubated with the antibody for 1 hour and then a FITC conjugated goat polyclonal antibody was used as the secondary. The cells were counterstaind with DAPI for the nucleus and Cell Tracker Blue for the cytoplasm.



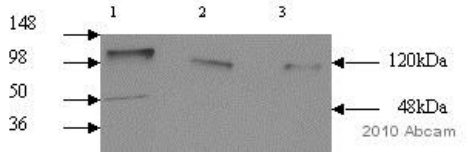
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-LAMP1 antibody [H4A3] - Drosophila Lysosome Marker (ab25630)

Ab25630 staining Human normal placenta. Staining is localized to the cell membrane.
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.



Immunocytochemistry/ Immunofluorescence - Anti-LAMP1 antibody [H4A3] - Drosophila Lysosome Marker (ab25630)

ab25630 staining LAMP1 in human HaCaT keratinocytes by Immunocytochemistry/ Immunofluorescence. Cells were fixed with acetone, permeabilized with ice cold acetone and blocking with 4% PBS, 0.4% BSA and goat serum was performed for 16 hours at 4^0C. Samples were incubated with primary antibody (1/100: in 10% blocking solution in PBS) for 1 hour at 23^0C. An Alexa Fluor ® 680-conjugated goat polyclonal to mouse IgG was used at dilution at 1/200 as secondary antibody. Alexafluor-680 signal is pseudocolored green in the image.



Western blot - Anti-LAMP1 antibody [H4A3] - Drosophila Lysosome Marker (ab25630)

All lanes : Anti-LAMP1 antibody [H4A3] (ab25630) at 1/10000 dilution

Lane 1 : Iron treated 3 month old liver at 20 µg
Lane 2 : Untreated 3 month old liver at 20 µg
Lane 3 : One month old untreated liver

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 45 kDa
Observed band size: 120,48 kDa
Additional bands at: 120 kDa (possible glycosylated form)

Exposure time: 5 minutes

WB image of LAMP1 (ab25630) on Mouse liver. Lanes were loaded 20 ug of Liver tissue lysate Lane 1. iron treated 3 month old liver, lane 2. untreated 3 month old liver, Lane 3. one month old untreated liver.

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