# abcam

# Product datasheet

# Anti-Lamin B1 antibody - Nuclear Envelope Marker ab16048



\*\*\*\* 108 Abreviews 1046 References 11 Images

#### Overview

**Immunogen** 

Product name Anti-Lamin B1 antibody - Nuclear Envelope Marker

**Description** Rabbit polyclonal to Lamin B1 - Nuclear Envelope Marker

Host species Rabbit

Tested applications Suitable for: ICC/IF, WB, IHC-P

**Species reactivity** Reacts with: Mouse, Rat, Human

**Predicted to work with:** Chicken, Pig, Xenopus laevis, Indian muntjac, Zebrafish

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

(Peptide available as ab16375)

Positive control ICC/IF: HepG2 cells WB: HeLa, PC12 and NIH/3T3 whole cell lysate. Wild type HAP1 whole cell

lysate. Wild type HAP1 nuclear lysate. Human Pancreatic cell line whole cell lysate. IHC-P: Human

liver tissue. Human infantile fibromatosis tissue.

General notes Lamin B1 and Lamin B antibodies are extremely useful as nuclear loading controls for use with

nuclear extracts. When using Lamin B1 antibodies as nuclear loading controls, be aware that in apoptotic cells Lamin B1 is cleaved (Kottke TJ et al.). Lamin B1 will also be removed from a nuclear prep if the nuclear membranes are spun out. This antibody was designed to be a nuclear

loading control however it has not yet been tested in appropriate lysates.

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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C or -

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80°C. Avoid freeze / thaw cycle.

Storage buffer

pH: 7.40

Preservative: 0.02% Sodium azide

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.

Purity Immunogen affinity purified

Primary antibody notes Lamin B1 and Lamin B antibodies are extremely useful as nuclear loading controls for use with

nuclear extracts. When using Lamin B1 antibodies as nuclear loading controls, be aware that in apoptotic cells Lamin B1 is cleaved (Kottke TJ et al.). Lamin B1 will also be removed from a nuclear prep if the nuclear membranes are spun out. This antibody was designed to be a nuclear

loading control however it has not yet been tested in appropriate lysates.

**Clonality** Polyclonal

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our Abpromise guarantee covers the use of ab16048 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	<b>★★★★★ (28)</b>	Use a concentration of 0.1 μg/ml.
WB	★★★★☆ (66)	Use a concentration of 0.1 µg/ml. Detects a band of approximately 68 kDa (predicted molecular weight: 66 kDa). We recommend <b>Goat anti-Rabbit lgG H&amp;L (IRDye® 800CW) preadsorbed (ab216773)</b> .
IHC-P	★★★★★ (5)	Use a concentration of 0.1 - 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

#### **Target**

Function Lamins are components of the nuclear lamina, a fibrous layer on the nucleoplasmic side of the

inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and  $% \left( 1\right) =\left( 1\right) \left( 1\right) \left($ 

may also interact with chromatin.

**Involvement in disease** Defects in LMNB1 are the cause of leukodystrophy demyelinating autosomal dominant adult-

onset (ADLD) [MIM:169500]. ADLD is a slowly progressive and fatal demyelinating

leukodystrophy, presenting in the fourth or fifth decade of life. Clinically characterized by early autonomic abnormalities, pyramidal and cerebellar dysfunction, and symmetric demyelination of

the CNS. It differs from multiple sclerosis and other demyelinating disorders in that

neuropathology shows preservation of oligodendroglia in the presence of subtotal demyelination

and lack of astrogliosis.

**Sequence similarities** Belongs to the intermediate filament family.

# Post-translational modifications

B-type lamins undergo a series of modifications, such as farnesylation and phosphorylation.

Increased phosphorylation of the lamins occurs before envelope disintegration and probably plays

a role in regulating lamin associations.

## **Cellular localization**

Nucleus inner membrane.

## **Images**



Western blot - Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048) All lanes: Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048) at 1 µg/ml

Lane 1 : HeLa whole cell lysate

Lane 2 : PC12 whole cell lysate

Lane 3 : NIH 3T3 whole cell lysate

Lysates/proteins at 10 µg per lane.

# **Secondary**

**All lanes :** Goat polyclonal to Rabbit lgG - H&L - Pre-Adsorbed (HRP) at 1/50000 dilution

Predicted band size: 66 kDa

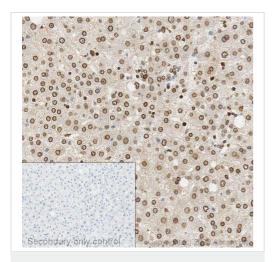
Blocking buffer: 3% Milk

Gel type: MOPS

Exposure Time: 2 minutes

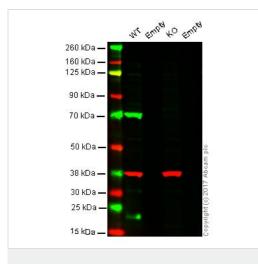
Observed band size: 73 kDa

Additional bands: 46 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048)

Immunohistochemical analysis of formalin-fixed paraffin-embedded human liver labelling lamin B1 with ab16048 at a concentration of 0.2µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32min with ULTRA cell conditioning solution (CC1 pH8.5). ab16048 anti lamin B1 antibody was incubated at 37°C for 16min. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



Western blot - Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)

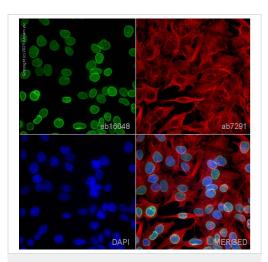
Lane 2: empty lane

Lane 3: KO HAP1 LMNB1 whole cell lysate (20 µg)

Lane 4: empty lane

**Lanes 1 - 4:** Merged signal (red and green). Green - ab16048 observed at 70 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab16048 was shown to specifically react with LMNB1 (Lamin B1) in wild type HAP1 cells. No band was observed when LMNB1 (Lamin B1) knockout samples were used. Ab16048 LMNB1 (Lamin B1) and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 0.1 µg per mL and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

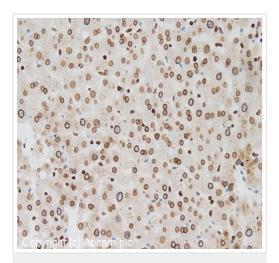


Immunocytochemistry/ Immunofluorescence - Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048)

ab16048 staining Lamin B1 in HepG2 cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 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 ab16048 at 0.1 µg/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).

Also suitable in cells fixed with 100% methanol (5 min).

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

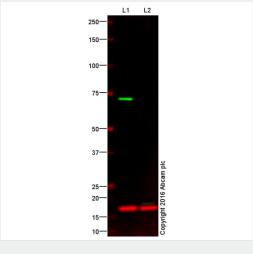


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048)

IHC image of Lamin B1 staining in Human normal Liver formalin fixed paraffin embedded tissue section\*, performed on a Leica Bond™ system using the standard protocol F. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with ab16048, 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-Lamin B1 antibody - Nuclear Envelope Marker (ab16048)

All lanes: Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048) at 1 µg/ml

Lane 1: Wild-type HAP1 nuclear lysate

Lane 2: Lamin B1 knockout HAP1 nuclear lysate

Lysates/proteins at 10 µg per lane.

## **Secondary**

**All lanes :** Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Performed under reducing conditions.

**Predicted band size:** 66 kDa **Observed band size:** 68 kDa

Lane 1: Wild-type HAP1 nuclear lysate (10 µg)

Lane 2: Lamin B1 knockout HAP1 nuclear lysate (10 µg)

**Lanes 1 and 2:** Green signal from target - ab16048 observed at 68 kDa. Red signal from loading control - **ab10799** observed at 18 kDa.

ab16048 was shown to specifically react with lamin B1 in wild-type HAP1 cells. No band was observed knockout samples were used. Wild-type and lamin B1 knockout samples were subjected to SDS-PAGE. ab16048 and ab10799 (loading control to histone H3 at 0.1µg/mL) were both incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10,000 dilution for 1 h at room temperature before imaging.



Western blot - Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048)

**All lanes :** Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048) at 1/1000 dilution

**Lane 1**: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate

**Lane 2 :** HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate with Mouse Lamin B1 peptide (ab16375) at 1  $\mu g/ml$ 

Lysates/proteins at 20 µg per lane.

## **Secondary**

**All lanes :** Alexa fluor goat polyclonal to Rabbit lgG at 1/10000 dilution

Performed under reducing conditions.

**Predicted band size:** 66 kDa **Observed band size:** 68-70 kDa



Western blot - Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048)

This image is courtesy of an anonymous Abreview

Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048) at 1/1000 dilution + Human Pancreatic cell line - whole cell lysate at 20  $\mu g$ 

# **Secondary**

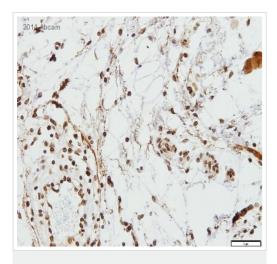
HRP conjugated goat anti-rabbit antibody at 1/2000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

**Predicted band size:** 66 kDa **Observed band size:** 68 kDa

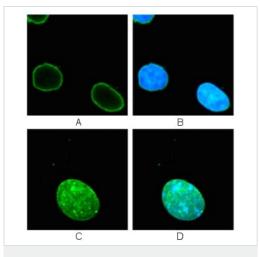
#### Exposure time: 30 seconds



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin B1 antibody -Nuclear Envelope Marker (ab16048)

This image is courtesy of an anonymous Abreview

ab16048 staining Lamin B1 in human infantile fibromatosis tissue sections by Immunohistochemistry (IHC-P - paraformaldehydefixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 1% FBS/BSA for 3 hours at room temperature; antigen retrieval was by heat mediation in Tris pH9. Samples were incubated with primary antibody (1/100 in TBS + 1% BSA + 1% FBS) for 16 hours. An undiluted HRP-conjugated goat anti-rabbit IgG polyclonal was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048)

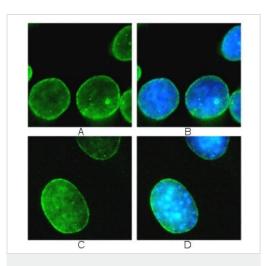
Image courtesy of Marilena Ciciarello and Patrizia Lavia, University 'La Sapienza' CNR, Italy Human and mouse cells stained with ab16048 (1/500). The cells were fixed and permeabilized in 4% formaldehyde, 0.2% Tritonm X100 for 10 minutes at room temperature, then washed 3x in PBS.

A: HeLa cells + ab16048 (green)

B: HeLa cells counterstained with DAPI (blue)

C: 3T3 cells + ab16048 (green)

D: 3T3 cells counterstained with DAPI (blue)



Immunocytochemistry/ Immunofluorescence - Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048)

Image courtesy of Marilena Ciciarello and Patrizia Lavia, University 'La Sapienza' CNR, Italy

Human and mouse cells stained with ab16048 (1/500). The cells were fixed in 100% methanol for 6 minutes at -20°C, then washed once in PBS.

A: HeLa cells + ab16048 (green)

B: HeLa cells counterstained with DAPI (blue)

C: 3T3 cells + ab16048 (green)

D: 3T3 cells counterstained with DAPI (blue)

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