Product datasheet

Anti-Lamin B1 antibody - Nuclear Envelope Marker
ab16048

Overview

Product name  Anti-Lamin B1 antibody - Nuclear Envelope Marker
Description  Rabbit polyclonal to Lamin B1 - Nuclear Envelope Marker
Host species  Rabbit
Specificity  Lamins do not appear to be universally distributed among different cell and tissue types. ab16048 has been shown to react with HeLa cells/lysates in Western blot and ICC. Other cell/tissue types have not been tested.

Tested applications

- Suitable for: ICC/IF, IHC-Fr, WB, IHC-P, IHC - Wholemount

Species reactivity

- Reacts with: Mouse, Rat, Human, Pig, Xenopus laevis, Indian muntjac
- Predicted to work with: Chicken, Zebrafish

Immunogen  Synthetic peptide conjugated to KLH derived from within residues 400 - 500 of Mouse Lamin B1. Read Abcam's proprietary immunogen policy (Peptide available as ab16375.)

Positive control  This antibody gave a positive signal in the following whole cell lysates: HeLa. This antibody gave a positive signal in the following Methanol fixed cell lines: HeLa. This antibody gave a positive signal in the following FFPE tissue: Human normal liver.

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.

Abcam recommended secondaries - Goat Anti-Rabbit HRP (ab205718) and Goat Anti-Rabbit Alexa Fluor® 488 (ab150077).

See other anti-rabbit secondary antibodies that can be used with this antibody.

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 -80°C. Avoid freeze / thaw cycle.
Storage buffer  
Preservative: 0.02% Sodium Azide  
Constituents: 1% BSA, PBS, pH 7.4

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

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.

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<tr>
<td>ICC/IF</td>
<td>🟢🟢🟢🟢</td>
<td>Use a concentration of 0.1 - 1 µg/ml.</td>
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<tr>
<td>IHC-Fr</td>
<td>🟢🟢🟢🟢</td>
<td>Use at an assay dependent concentration.</td>
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| WB          | 🟢🟢🟢🟢   | Use a concentration of 0.1 µg/ml. Detects a band of approximately 68 kDa (predicted molecular weight: 66 kDa).  
We recommend Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773). |
| IHC-P       | 🟢🟢🟢🟢   | Use a concentration of 0.1 - 1 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. |
| IHC - Wholemount | 🟢🟢🟢   | Use at an assay dependent concentration. PubMed: 25368174 |

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 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**

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.

Human and mouse cells stained with ab16048 (1/500). The cells were fixed and permeabilized in 4% formaldehyde, 0.2% Triton 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)
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded 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 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 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)

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 whole cell lysate

**Lane 2**: Hela whole cell lysate with Mouse Lamin B1 peptide (ab16375) at 1 µg/ml

Lysates/proteins at 20 µg per lane.

**Secondary**

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

Performed under reducing conditions.

**Predicted band size**: 66 kDa

**Observed band size**: 68-70 kDa *why is the actual band size different from the predicted?*

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

ICC/IF image of ab16048 stained human HeLa cells. The cells were methanol fixed (5 min) and incubated with the antibody (ab16048, 1µg/ml) for 1h at room temperature. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Image-iT™FX Signal Enhancer was used as the primary blocking agent, 5% BSA (in TBS-T) was used for all other blocking steps. DAPI was used to stain the cell nuclei (blue). Alexa Fluor® 594 WGA was used to label plasma membranes (red).
Anti-Lamin B1 antibody - Nuclear Envelope Marker (ab16048) at 1/1000 dilution + Pancreatic cell line - whole cell lysate at 20 µg

**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 *why is the actual band size different from the predicted?*

**Exposure time:** 30 seconds

ab16048 staining Lamin B1 in human infantile fibromatosis tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, 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.
ab16048 staining Lamin B1 in Pig PAE cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with formaldehyde and blocked with 3% serum for 1 hour at room temperature. Samples were incubated with primary antibody (1/100 in PBS + 1% BSA) for 1 hour. An Alexa Fluor® 488-conjugated donkey anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

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

All lanes : Pig PAE whole cell lysate

Secondary

All lanes : HRP-conjugated goat anti-rabbit IgG polyclonal at 1/2000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 66 kDa

Observed band size: 65 kDa

why is the actual band size different from the predicted?

Exposure time: 3 minutes
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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