# abcam

## Product datasheet

# Anti-Lamin B1 antibody [EPR8985(B)] - BSA and Azide free ab220797





RabMAb

**16 References** 13 Images

#### Overview

Product name Anti-Lamin B1 antibody [EPR8985(B)] - BSA and Azide free

**Description** Rabbit monoclonal [EPR8985(B)] to Lamin B1 - BSA and Azide free

Host species Rabbit

Tested applications
Suitable for: ICC/IF, IP, WB, IHC-P
Species reactivity
Reacts with: Mouse, Rat, Human

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

Positive control WB: HAP1, HeLa, Jurkat, Molt4, Y79, Caco 2, C6, Raw264.7, PC-12 and NIH/3T3 cell lysates.

Mouse brain, heart, kidney and spleen; and rat brain, heart and spleen lysates. IHC-P: Human colon, liver and transitional cell carcinoma of the bladder tissues. ICC/IF: Ramos cells, HAP1-

LMNB1 cells.

**General notes** ab220797 is the carrier-free version of **ab133741**.

Our <u>carrier-free</u> antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for

increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our <u>conjugation kits</u> for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar<sup>®</sup> Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar<sup>®</sup> is a trademark of Fluidigm Canada Inc.

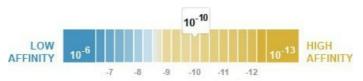
Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**<sup>®</sup> **patents**.

#### **Properties**

Form Liquid

**Storage instructions** Shipped at 4°C. Store at +4°C. Do Not Freeze.

**Dissociation constant (K<sub>D</sub>)**  $K_D = 1.95 \times 10^{-10} M$ 



Learn more about K<sub>D</sub>

Storage buffer pH: 7.20

Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal
Clone number EPR8985(B)

**Isotype** IgG

#### **Applications**

#### The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab220797 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		Use at an assay dependent concentration.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 70 kDa (predicted molecular weight: 66 kDa).
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.  See IHC antigen retrieval protocols.

#### **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

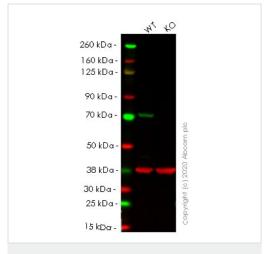
B-type lamins undergo a series of modifications, such as farnesylation and phosphorylation. Increased phosphorylation of the lamins occurs before envelope disintegration and probably plays

a role in regulating lamin associations.

**Cellular localization** 

Nucleus inner membrane.

#### **Images**



Western blot - Anti-Lamin B1 antibody

[EPR8985(B)] - BSA and Azide free (ab220797)

**All lanes :** Anti-Lamin B1 antibody [EPR8985(B)] - Nuclear Envelope Marker (**ab133741**) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: LMNB1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

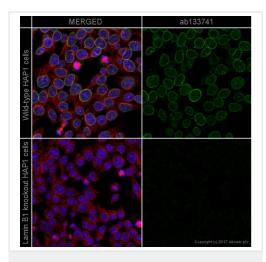
Performed under reducing conditions.

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

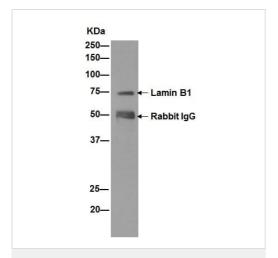
This data was developed using the same antibody clone in a different buffer formulation (ab133741).

**Lanes 1-2:** Merged signal (red and green). Green - <u>ab133741</u> observed at 66-70 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (<u>ab8245</u>) observed at 37 kDa.

ab133741 was shown to react with Lamin B1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab255404 (knockout cell lysate ab263825) was used. Wild-type HeLa and LMNB1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab133741 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Lamin B1 antibody [EPR8985(B)] - BSA and Azide free (ab220797)



Immunoprecipitation - Anti-Lamin B1 antibody [EPR8985(B)] - BSA and Azide free (ab220797)

<u>ab133741</u> staining Lamin B1 in wild-type HAP1 cells (top panel) and Lamin B1 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% 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 with <u>ab133741</u> at 1μg/ml dilution and <u>ab195889</u> at 1/250 dilution (shown in pseudo-color red) overnight at +4°C. The cells were then incubated with <u>ab150081</u> (Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488)) at 1/1000 dilution for 1 hour. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

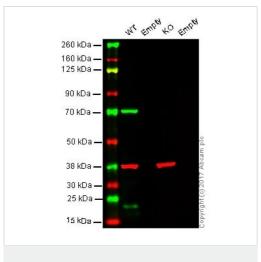
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab133741</u>).

<u>ab133741</u> (purified) at 1/20 immunoprecipitating Lamin
B1 in Jurkat cells (Lane 1). For western blotting, <u>ab133741</u> was
used at 1/1000 dilution and an HRP-conjugated goat anti-rabbit lgG
was used as the secondary antibody (1/1500).

Blocking buffer and concentration: 5% NFDM/TBST.

Diluting buffer and concentration: 5% NFDM /TBST.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab133741).



Western blot - Anti-Lamin B1 antibody

[EPR8985(B)] - BSA and Azide free (ab220797)

Lanes 1 & 3: Anti-Lamin B1 antibody [EPR8985(B)] - Nuclear Envelope Marker (ab133741) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate at 20 µg

Lanes 2 & 4: Empty

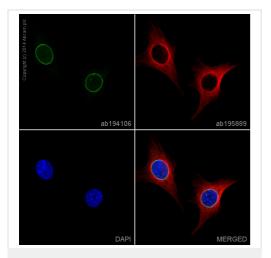
Lane 3: LMNB1 knockout HAP1 whole cell lysate at 20 µg

Predicted band size: 66 kDa

This data was developed using the same antibody clone in a different buffer formulation (<u>ab133741</u>).

**Lanes 1 - 4:** Merged signal (red and green). Green - <u>ab133741</u> observed at 70 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab133741 was shown to specifically react with Lamin B1 in wild type HAP1 cells. No band was observed when knockout samples were used. Wild-type and Lamin B1 knockout samples were subjected to SDS-PAGE. Ab133741 and ab8245 (Mouse anti GAPDH loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed ab216773 and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ab216776 secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.

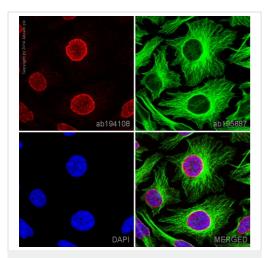


Immunocytochemistry/ Immunofluorescence - Anti-Lamin B1 antibody [EPR8985(B)] - BSA and Azide free (ab220797)

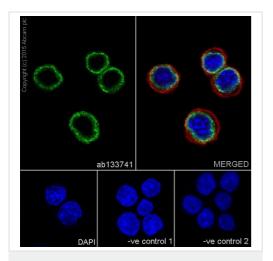
Clone EPR8985(B) (ab220797) has been successfully conjugated by Abcam. This image was generated using Anti-Lamin B1 antibody [EPR8985(B)] - Nuclear Envelope Marker (Alexa Fluor® 488). Please refer to ab194106 for protocol details.

ab194106 staining Lamin B1 in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilised in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab194106 at 1/100 dilution (shown in green) and ab195889, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594, shown in red) at 1/250 dilution overnight at +4°C.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-Lamin B1 antibody [EPR8985(B)] - BSA and Azide free (ab220797)



Immunocytochemistry/ Immunofluorescence - Anti-Lamin B1 antibody [EPR8985(B)] - BSA and Azide free (ab220797)

Clone EPR8985(B) (ab220797) has been successfully conjugated by Abcam. This image was generated using Anti-Lamin B1 antibody [EPR8985(B)] - Nuclear Envelope Marker (Alexa Fluor® 647). Please refer to <u>ab194108</u> for protocol details.

<u>ab194108</u> staining Lamin B1 in HeLa cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% 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 <u>ab194108</u> at a 1/100 dilution (shown in red) and <u>ab195887</u>, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at a 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

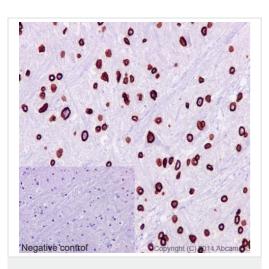
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized Ramos (Human Burkitt's lymphoma cell line) cells labeling Lamin B1 with <a href="mailto:ab133741">ab133741</a> at 1/100 dilution, followed by Goat anti-rabbit lgG (Alexa Fluor® 488) (<a href="mailto:ab150077">ab150077</a>) secondary antibody at 1/200 dilution (green). Nuclear envelope staining on Ramos cell line is observed. The nuclear counter stain is DAPI (blue). Tubulin is detected with <a href="mailto:ab7291">ab7291</a> (anti-Tubulin mouse mAb) at 1/500 dilution and <a href="mailto:ab150120">ab150120</a> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution (red).

The negative controls are as follows:

-ve control 1: <u>ab133741</u> at 1/100 dilution followed by <u>ab150120</u> (AlexaFluor®594 Goat anti-Mouse secondary) at 1/500 dilution. -ve control 2: <u>ab7291</u> (anti-Tubulin mouse mAb) at 1/500 dilution followed by <u>ab150077</u> (Alexa Fluor®488 Goat Anti-Rabbit lgG H&L) at 1/200 dilution.

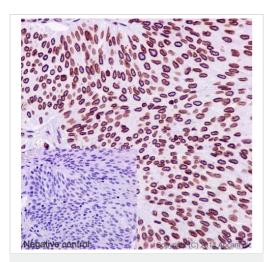
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab133741</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin B1 antibody
[EPR8985(B)] - BSA and Azide free (ab220797)

Immunohistochemical staining of paraffin embedded Mouse
Cerebral cortex with purified <u>ab133741</u> at a working dilution of
1/300. The secondary antibody used is a HRP polymer for rabbit
lgG. Nuclear envelope staining on neuron cells of Cerebral cortex
tissue is observed. The sample is counter-stained with hematoxylin.
Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0.
PBS was used instead of the primary antibody as the negative
control, and is shown in the inset.

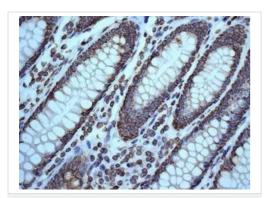
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab133741</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin B1 antibody
[EPR8985(B)] - BSA and Azide free (ab220797)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human transitional cell carcinoma of the bladder tissue labeling Lamin B1 with purified <u>ab133741</u> at 1/300. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit lgG was used as the secondary antibody. Negative control using PBS instead of primary antibody. Counterstained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab133741</u>).



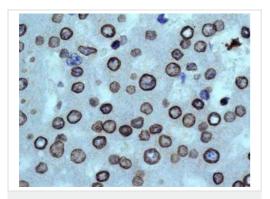
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin B1 antibody

[EPR8985(B)] - BSA and Azide free (ab220797)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colon tissue labelling Lamin B1 with unpurified <u>ab133741</u> at 1/250.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab133741**).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



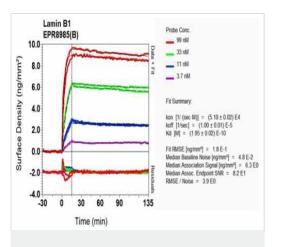
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin B1 antibody

[EPR8985(B)] - BSA and Azide free (ab220797)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling Lamin B1 with unpurified <u>ab133741</u> at 1/250.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab133741</u>).

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

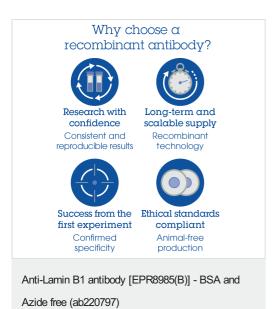


Ol-RD Scanning - Anti-Lamin B1 antibody [EPR8985(B)] - BSA and Azide free (ab220797)

Equilibrium disassociation constant ( $K_D$ ) Learn more about  $K_D$ 

### Click here to learn more about K<sub>D</sub>

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab133741</u>).



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