

Product datasheet

Anti-Lamin B1 antibody [EPR22165-121] ab229025

KO VALIDATED Recombinant RabMAb

★★★★☆ 5 Abreviews 7 References 16 Images

Overview

Product name	Anti-Lamin B1 antibody [EPR22165-121]
Description	Rabbit monoclonal [EPR22165-121] to Lamin B1
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), WB, IHC-P, ICC/IF, IP, mIHC
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HAP1, HepG2, Caco-2, RAW 264.7, PC-12 and NIH/3T3 whole cell lysate; Human lymph node tissue; Mouse brain and heart tissue lysate; Rat brain, heart and spleen tissue lysate. IHC-P: Human breast and liver tissue; Mouse liver tissue, Rat cerebrum tissue. mIHC: Human liver tissue. ICC/IF: HeLa, HAP1, and NIH/3T3 cells. Flow: HeLa, HAP1 and NIH/3T3 cells. IP: NIH/3T3 and HeLa whole cell lysate.
General notes	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production <p>For more information see here.</p> <p>Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb[®] patents.</p>

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 long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR22165-121
Isotype	IgG

Applications

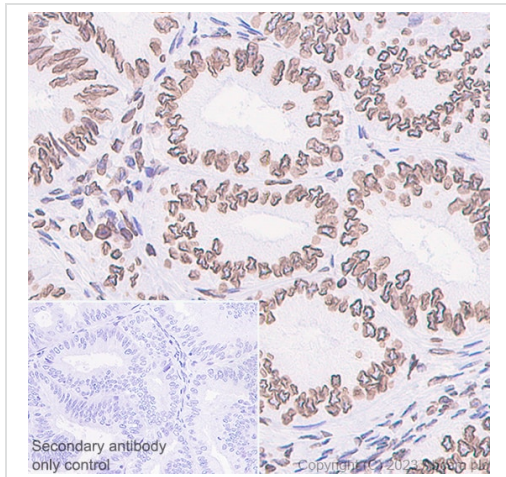
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab229025 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
WB	★★★★★ (5)	1/1000. Detects a band of approximately 70 kDa (predicted molecular weight: 66 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Heat mediated antigen retrieval using ab93684 (Tris/EDTA buffer, pH 9.0).
ICC/IF		1/1000.
IP		1/30.
mlHC		1/4000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 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 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.

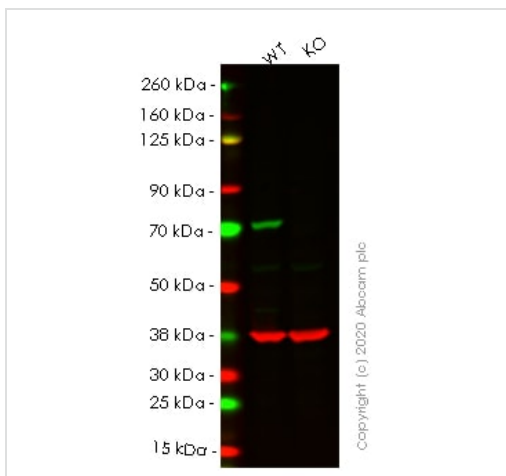
Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

Immunohistochemical analysis of paraffin-embedded human endometrial carcinoma stained for Lamin B1 using ab229025 at 1/2000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP polymer). Nuclear envelope staining on human endometrial carcinoma is observed. Counterstained with hematoxylin.

Heat mediated antigen retrieval was performed using [ab93684](#) (Tris/EDTA buffer, pH 9.0). The section was incubated with ab229025 at 4°C overnight



Western blot - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

All lanes : Anti-Lamin B1 antibody [EPR22165-121] (ab229025) 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.

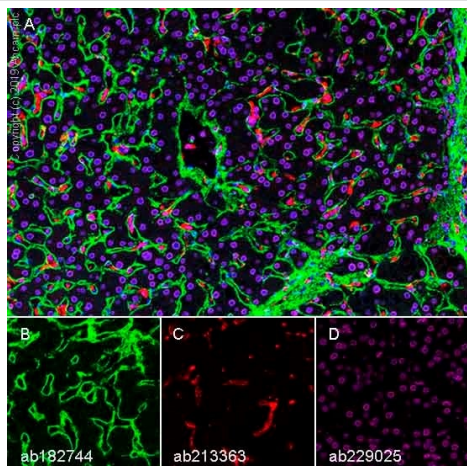
Predicted band size: 66 kDa

Observed band size: 66-70 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab229025 observed at 66-70 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control ([ab8245](#)) observed at 37 kDa.

ab229025 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. ab229025 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 IgG H&L (IRDye®800CW) preadsorbed ([ab216773](#)) and Goat anti-Mouse

IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Multiplex immunohistochemistry - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

Multiplex immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue.

Panel A: Merged staining of Collagen VI (**ab182744**; green), anti-CD68 (**ab213363**; red) and anti-Lamin B1 (ab229025; magenta).

Panel B: Anti-Collagen VI (green) stained on extracellular matrix.

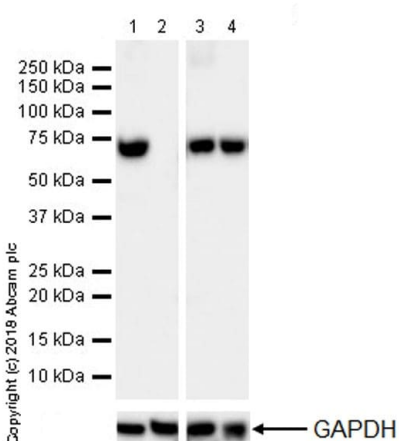
Panel C: Anti-CD68 (red) stained on Kupffer cells.

Panel D: Anti-Lamin B1 (magenta) stained on nuclear envelope.

Key protocol steps: The section was incubated in three rounds of staining with **ab182744** (1/1000 dilution), **ab213363** (1/1000 dilution) and ab229025 (1/4000 dilution) for 30 mins at room temperature. Each round was followed by tyramide signal amplification with the appropriate fluorophore. Heat mediated antigen retrieval was used (Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins after every round of antibody/fluorophore staining.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

DAPI was used as a nuclear counter stain. A ready-to-use anti-Rabbit and Mouse Polymer HRP was used as a secondary.



Western blot - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

All lanes : Anti-Lamin B1 antibody [EPR22165-121] (ab229025) at 1/5000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate

Lane 2 : Lamin B1 knockout HAP1 whole cell lysate

Lane 3 : HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate

Lane 4 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate

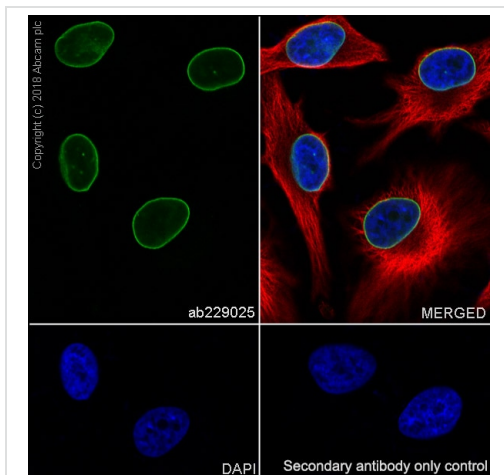
Lysates/proteins at 20 µg per lane.

Predicted band size: 66 kDa

Observed band size: 70 kDa

Exposure time: 8 seconds

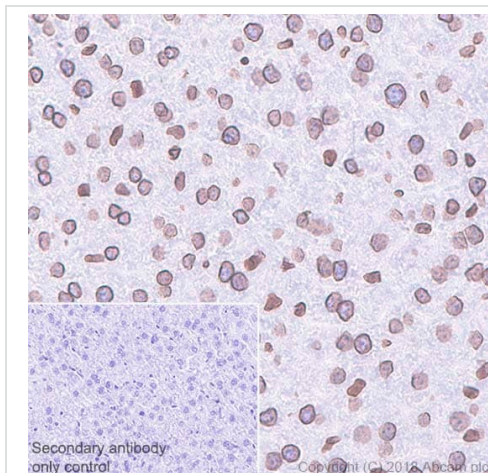
ab229025 was shown to specifically react with Lamin B1 in wild-type HAP1 cells as signal was lost in Lamin B1 knockout cells. Wild-type and Lamin B1 knockout samples were subjected to SDS-PAGE. ab229025 and **ab181602** (Rabbit anti-GAPDH loading control) were incubated 1 hour at room temperature at 1/1000 dilution and 1/200,000 dilution respectively. Blots were developed with Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) secondary antibody at 1/100,000 dilution for 1 hour at room temperature before imaging. The blot was developed on a BIO-RAD® ChemiDoc™ MP instrument using the ECL technique.



Immunocytochemistry/ Immunofluorescence - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling Lamin B1 (Green) with ab229025 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution. Confocal image showing nuclear membranous staining in HeLa cells. Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) (**ab195889**) was used as a counterstain at 1/200 dilution. The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Alexa Fluor® 488 Goat anti-Rabbit secondary (**ab150077**) at 1/1000 dilution.

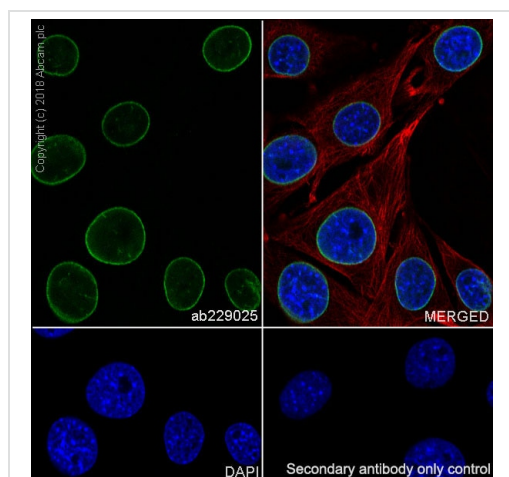


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

Immunohistochemical analysis of paraffin-embedded mouse liver tissue stained for Lamin B1 using ab229025 at 1/2000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear envelope staining on mouse liver (PMID: 19925772) is observed. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

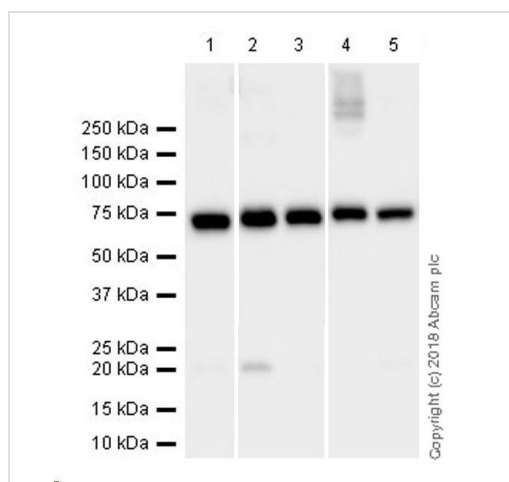
Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0).



Immunocytochemistry/ Immunofluorescence - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryonic fibroblast cell line) cells labeling Lamin B1 (Green) with ab229025 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) ([ab150077](#)) secondary antibody at 1/1000 dilution. Confocal image showing nuclear membranous staining in NIH/3T3 cells. Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) ([ab195889](#)) was used as a counterstain at 1/200 dilution. The nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Alexa Fluor®488 Goat anti-Rabbit secondary ([ab150077](#)) at 1/1000 dilution.



Western blot - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

All lanes : Anti-Lamin B1 antibody [EPR22165-121] (ab229025) at 1/1000 dilution

Lane 1 : Human lymph node tissue lysate

Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 3 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

Lane 4 : Caco-2 (Human colorectal adenocarcinoma cell line) whole cell lysate

Lane 5 : Mouse brain tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

Lane 1 : VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)) at 1/100000 dilution

Lanes 2-5 : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Predicted band size: 66 kDa

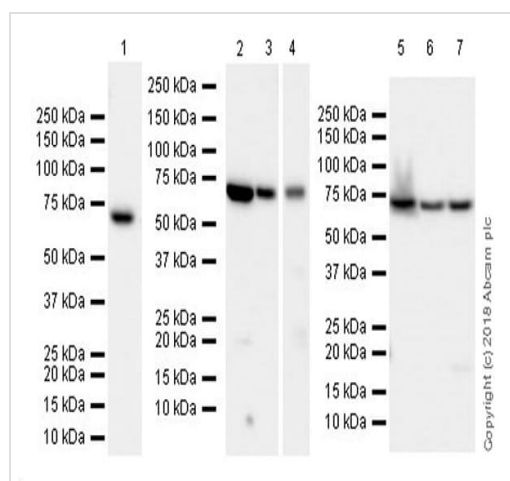
Observed band size: 70 kDa

Blocking/diluting buffer and concentration: 5% NFDM/TBST.

Exposure time:

Lane 1:3 seconds;

Lanes 2,3,4 and 5:10 seconds.



Western blot - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

All lanes : Anti-Lamin B1 antibody [EPR22165-121] (ab229025) at 1/1000 dilution

Lane 1 : Mouse heart tissue lysate

Lane 2 : Rat brain tissue lysate

Lane 3 : Rat heart tissue lysate

Lane 4 : Rat spleen tissue lysate

Lane 5 : RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate

Lane 6 : PC-12 (Rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 7 : NIH/3T3 (Mouse embryo fibroblast cell line) whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) ([ab97051](#)) at 1/100000 dilution

Predicted band size: 66 kDa

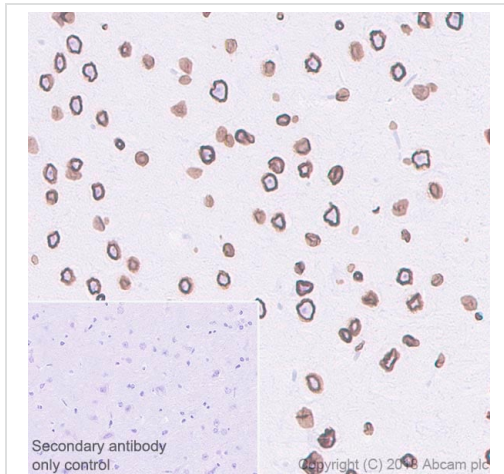
Observed band size: 70 kDa

Blocking/diluting buffer and concentration: 5% NFDM/TBST.

Exposure time:

Lane 1:10 seconds; Lanes 2 and 3: 59 seconds; Lane 4:3 seconds;

Lanes 5,6 and 7:6 seconds.

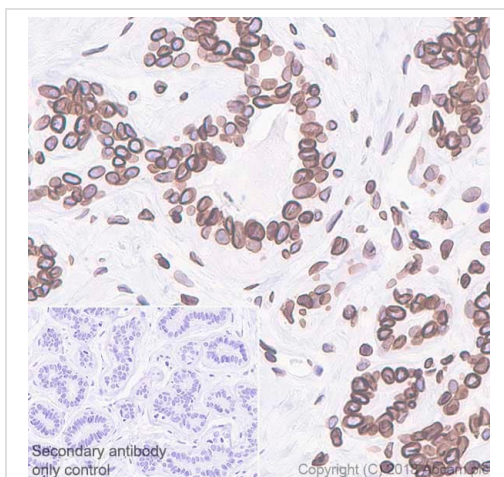


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

Immunohistochemical analysis of paraffin-embedded rat cerebrum tissue stained for Lamin B1 using ab229025 at 1/2000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear envelope staining on rat cerebrum (PMID: 26469707, PMID: 19925772) is observed. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval was performed using [ab93684](#) (Tris/EDTA buffer, pH 9.0).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

Immunohistochemical analysis of paraffin-embedded human breast tissue stained for Lamin B1 using ab229025 at 1/2000 dilution, followed by a ready to use Goat Anti-Rabbit IgG H&L (HRP). Nuclear envelope staining on human breast (PMID: 26469707) is observed. Counterstained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) ready to use.

Heat mediated antigen retrieval was performed using [ab93684](#) (Tris/EDTA buffer, pH 9.0).



Immunoprecipitation - Anti-Lamin B1 antibody
[EPR22165-121] (ab229025)

Lamin B1 was immunoprecipitated from 0.35 mg HeLa (human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab229025 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab229025 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)), was used for detection at 1/5000 dilution.

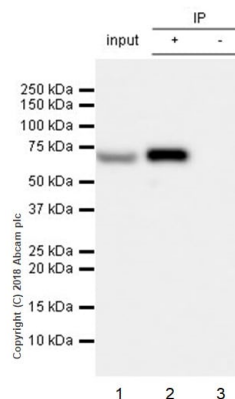
Lane 1: HeLa whole cell lysate 10 µg (input).

Lane 2: ab229025 IP in HeLa whole cell lysate (+).

Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of ab229025 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 5 seconds.

A very weak band around 66 kDa detected in lane 3 is due to spill over from lane2 (+).



Immunoprecipitation - Anti-Lamin B1 antibody
[EPR22165-121] (ab229025)

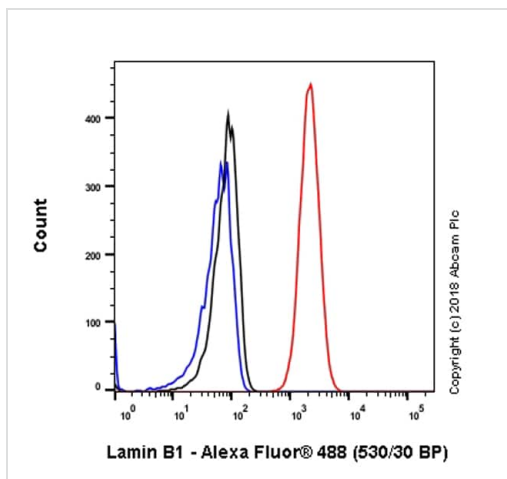
Lamin B1 was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryo fibroblast cell line) whole cell lysate with ab229025 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab229025 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) ([ab131366](#)), was used for detection at 1/5000 dilution.

Lane 1: NIH/3T3 whole cell lysate 10 µg (input).

Lane 2: ab229025 IP in NIH/3T3 whole cell lysate (+).

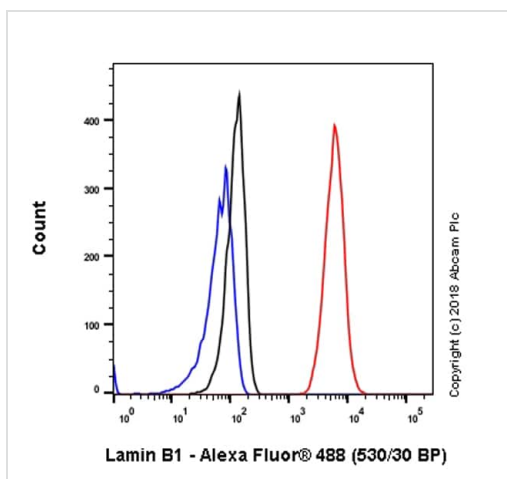
Lane 3: Rabbit monoclonal IgG ([ab172730](#)) instead of ab229025 in NIH/3T3 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.
Exposure time: 5 seconds.



Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cell line labeling Lamin B1 with ab229025 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG isotype control (**ab172730**) (Black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor[®] 488, **ab150077**) was used as the secondary antibody at 1/2000 dilution.

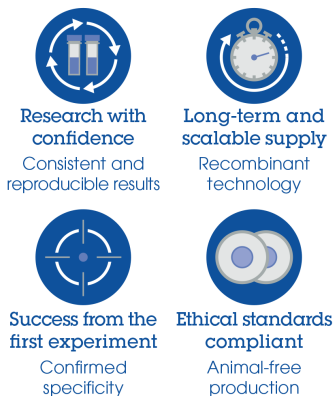
Flow Cytometry (Intracellular) - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)



Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cell line labeling Lamin B1 with ab229025 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG isotype control (**ab172730**) (Black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). Goat anti rabbit IgG (Alexa Fluor[®] 488, **ab150077**) was used as the secondary antibody at 1/2000 dilution.

Flow Cytometry (Intracellular) - Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

Why choose a recombinant antibody?



Anti-Lamin B1 antibody [EPR22165-121] (ab229025)

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- Response to your inquiry within 24 hours

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