

### **Product datasheet**

## Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free ab216074

KO VALIDATED Recombinant RabMAb

7 References 15 Images

Overview	
Product name	Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free
Description	Rabbit monoclonal [EPR4100] to Lamin A + Lamin C - BSA and Azide free
Host species	Rabbit
Specificity	The antibody recognizes full length Lamin A/C and the cleaved large unit. We have data to indicate that this antibody gives non-specific staining in IHC with mouse tissues. Based on this we believe the antibody is not suitable for use with mouse samples, as there will be non-specific staining.
Tested applications	Suitable for: WB, IP, IHC-P, Flow Cyt (Intra), ICC/IF
Species reactivity	Reacts with: Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HepG2, HACAT and SH-SY5Y cell lysates. IHC-P: Human brain, liver, cervix carcinoma, breast carcinoma, urothelial carcinoma and colonic carcinoma tissues. ICC/IF HeLa cells. Flow Cyt (intra): HeLa cells.
General notes	ab216074 is the carrier-free version of <u>ab108595</u> .
	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.
	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply

- Animal-free production

For more information see here.

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

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with these species. Please contact us for more information.

#### Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR4100
lsotype	lgG

#### **Applications**

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab216074 in the following tested applications.

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

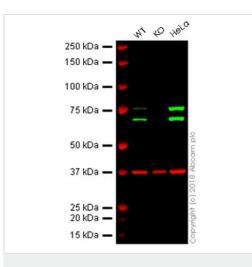
Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Predicted molecular weight: 63,74 kDa.
IP		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See <b>IHC antigen retrieval protocols</b> .
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.

Function

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

Tissue specificity	<ul> <li>inner nuclear membrane, which is thought to provide a framework for the nuclear envelope and may also interact with chromatin. Lamin A and C are present in equal amounts in the lamina of mammals. Play an important role in nuclear assembly, chromatin organization, nuclear membrane and telomere dynamics.</li> <li>Prelamin-A/C can accelerate smooth muscle cell senescence. It acts to disrupt mitosis and induce DNA damage in vascular smooth muscle cells (VSMCs), leading to mitotic failure, genomic instability, and premature senescence.</li> <li>In the arteries, prelamin-A/C accumulation is not observed in young healthy vessels but is prevalent in medial vascular smooth muscle celle (VSMCs) from aged individuals and in atherosclerotic lesions, where it often colocalizes with senescent and degenerate VSMCs.</li> <li>Prelamin-A/C expression increases with age and disease. In normal aging, the accumulation of prelamin-A/C is caused in part by the down-regulation of ZMPSTE24/FACE1 in response to oxidative stress.</li> </ul>
Involvement in disease	Defects in LMNA are the cause of Emery-Dreifuss muscular dystrophy type 2 (EDMD2) [MM:181350]. A degenerative myopathy characterized by weakness and atrophy of muscle without involvement of the nervous system, early contractures of the elbows, Achiles tendons and spine, and cardiomyopathy associated with cardiac conduction defects. Defects in LMNA are the cause of cardiomyopathy dilated type 1A (CMD1A) [MIM:115200]. Dilated cardiomyopathy is a disorder characterized by ventricular dilation and impaired systolic function, resulting in congestive heart failure and arrhythmia. Patients are at risk of premature death. Defects in LMNA are the cause of familial partial lipodystrophy type 2 (FPLD2) [MIM:151660]; also known as familial partial lipodystrophy Dunnigan type. A disorder characterized by the loss of subcutaneous adipose tissue in the lower parts of the body (limbs, buttocks, trunk). It is accompanied by an accumulation of adipose tissue in the face and neck causing a double chin, fat neck, or cushingoid appearance. Adipose tissue may also accumulate in the axillae, back, labia majora, and intraabdominal region. Affected patients are insulin-resistant and may develop glucose intolerance and diabetes mellitus after age 20 years, hypertriglyceridemia, and low levels of high density lipoprotein cholesterol. Defects in LMNA are the cause of limb-girdle muscular dystrophy type 1B (LGMD1B) [MM:150011]. LGMD1B is an autosomal dominant degenerative myopathy with age-related atrioventricular cardiac conduction disturbances, dilated cardiomyopathy, and the absence of early contractures. LGMD1B is characterized by slowly progressive skeletal muscle weakness of the hip and shoulder girdles. Muscle biopsy shows mild dystrophic changes. Defects in LMNA are the cause of Charcot-Marie-Tooth disease, the most common inherited disorder of the peripheral nervous system. Charcot-Marie-Tooth disease is classified in two main groups on the basis of electrophysiologic properties and histopathology: primary p

	<ul> <li>(CMDHH) [MIM:212112]. A disorder characterized by the association of genital anomalies, hypergonadotropic hypogonadism and dilated cardiomyopathy. Patients can present other variable clinical manifestations including mental retardation, skeletal anomalies, scleroderma-like skin, graying and thinning of hair, osteoporosis. Dilated cardiomyopathy is characterized by ventricular dilation and impaired systolic function, resulting in congestive heart failure and arrhythmia.</li> <li>Defects in LMNA are the cause of mandibuloacral dysplasia with type A lipodystrophy (MADA) [MIM:248370]. A disorder characterized by mandibular and clavicular hypoplasia, acroosteolysis, delayed closure of the cranial suture, progeroide appearance, partial alopecia, soft tissue calcinosis, joint contractures, and partial lipodystrophy with loss of subcutaneous fat from the extremities. Adipose tissue in the face, neck and trunk is normal or increased.</li> <li>Defects in LMNA are a cause of lethal tight skin contracture syndrome (LTSCS) [MIM:275210]; also known as restrictive dermopathy (RD). Lethal tight skin contracture syndrome is a rare disorder mainly characterized by intrauterine growth retardation, tight and rigid skin with erosions, prominent superficial vasculature and epidermal hyperkeratosis, facial features (small mouth, small pinched nose and micrognathia), sparse/absent eyelashes and eyebrows, mineralization defects of the skull, thin dysplastic clavicles, pulmonary hypoplasia, multiple joint contractures and an early neonatal lethal course. Liveborn children usually die within the first week of life. The overall prevalence of consanguineous cases suggested an autosomal recessive inheritance. Defects in LMNA are the cause of heart-hand syndrome Slovenian type (HHS-Slovenian) [MIM:610140]. Heart-hand syndrome (HHS) is a clinically and genetically heterogeneous disorder characterized by the co-occurrence of a congenital cardiac disease and limb malformations. Defects in LMNA are the cause of muscular dyst</li></ul>
Sequence similarities	Belongs to the intermediate filament family.
Post-translational modifications	Increased phosphorylation of the lamins occurs before envelope disintegration and probably plays a role in regulating lamin associations.
	Proteolytic cleavage of the C-terminal of 18 residues of prelamin-A/C results in the production of lamin-A/C. The prelamin-A/C maturation pathway includes farnesylation of CAAX motif, ZMPSTE24/FACE1 mediated cleavage of the last three amino acids, methylation of the C-terminal cysteine and endoproteolytic removal of the last 15 C-terminal amino acids. Proteolytic cleavage requires prior farnesylation and methylation, and absence of these blocks cleavage. Sumoylation is necessary for the localization to the nuclear envelope. Farnesylation of prelamin-A/C facilitates nuclear envelope targeting.
Cellular localization	Nucleus. Nucleus envelope. Farnesylation of prelamin-A/C facilitates nuclear envelope targeting and subsequent cleaveage by ZMPSTE24/FACE1 to remove the farnesyl group produces mature lamin-A/C, which can then be inserted into the nuclear lamina. EMD is required for proper localization of non-farnesylated prelamin-A/C.



Western blot - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074)

**All lanes :** Anti-Lamin A + Lamin C antibody [EPR4100] - Nuclear Envelope Marker (<u>ab108595</u>) at 1/10000 dilution

Lane 1 : Wild-type HAP1 whole cell lysate Lane 2 : Lamin A/C knockout HAP1 whole cell lysate Lane 3 : HeLa whole cell lysate

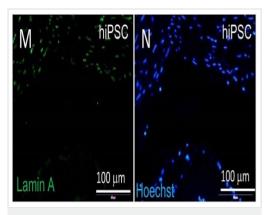
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 63,74 kDa Additional bands at: 64 kDa (possible cleavage fragment), 76 kDa (possible cleavage fragment)

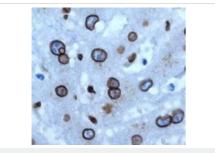
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108595**).

**ab108595** was shown to specifically react with Lamin A + C (LMNA) in wild type HAP1 cells. No band was observed when Lamin A + C (LMNA) knockout samples were used. Wild-type and Lamin A + C (LMNA) knockout samples were subjected to SDS-PAGE. The membrane was blocked for an hour using 5% milk before **ab108595** and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at a 1/10000 and 1/20000 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 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074)

Kanemura et al PLoS One. 2014 Jan 14;9(1):e85336. doi: 10.1371/journal.pone.0085336. eCollection 2014. Fig 6.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074)

# Histological analyses of hiPSCs (Human induced pluripotent stem cell) transplanted into the subretinal space of nude rats.

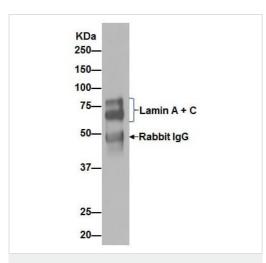
Eye balls were excised from a nude rat 7 weeks after subretinal transplantation with  $1 \times 10^4$  hiPSCs. Transplanted tissues were fixed with 4% paraformaldehyde. Paraffin embedded tissue sections were stained with haematoxylin/eosin. Then, the paraffin sections were deparaffinized with xylene and sequential 100%, 95%, 80%, 70% ethanol treatments for 5 min each. The sections were treated with 10 mM citric acid (pH 6) at 95°C for 50 min followed by permeation with 0.4% Triton-X in PBS at room temperature for 30 min.

The deparaffinized sections were stained with <u>ab108595</u> (Panel M), Hoechst 33258 (Panel N).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab108595</u>).

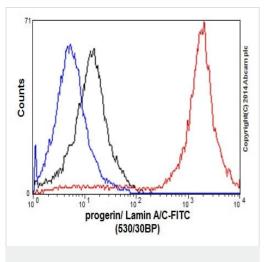
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human brain tissue labeling Lamin A + C with <u>ab108595</u> at 1/250 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab108595</u>).



**ab108595** (purified) at 1/30 immunoprecipitating Lamin A + C in HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate. For western blotting, a peroxidase-conjugated goat antirabbit lgG (H+L) was used as the secondary antibody (1/1000). Blocking/Dilution 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 (**ab108595**).

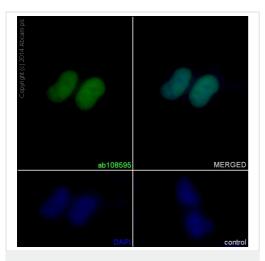
Immunoprecipitation - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074)



Flow Cytometry (Intracellular) - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074) Intracellular Flow Cytometry analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Lamin A + C with purified <u>ab108595</u> at 1/110 (red). Cells were fixed with 2% paraformaldehyde. A FITC-conjugated goat anti-rabbit lgG (1/150) was used as the secondary antibody.

Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabeled control, cells without incubation with primary and secondary antibodies.

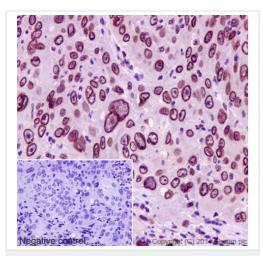
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab108595</u>).



Immunocytochemistry/ Immunofluorescence - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074) Immunocytochemistry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Lamin A + C (green) with purified <u>ab108595</u> at 1/500. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. <u>ab150077</u>, an Alexa Fluor<sup>®</sup> 488-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: Primary antibody (1/500) and secondary antibody **<u>ab150120</u>**, an Alexa Fluor<sup>®</sup> 594-conjugated goat anti-mouse IgG (1/500).

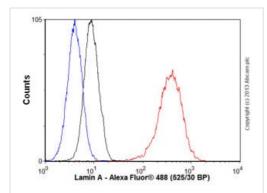
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108595**).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervix carcinoma tissue labeling Lamin A + C with purified <u>ab108595</u> at 1/250. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9. A prediluted HRP-polymer conjugated anti-rabbit IgG was used as the secondary antibody. Counterstained with hematoxylin.

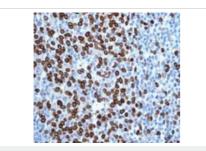
Negative control using PBS instead of primary antibody (inset).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108595**).



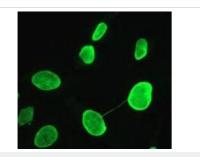
Flow Cytometry (Intracellular) - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074) Overlay histogram showing HeLa (Human epithelial cell line from cervix adenocarcinoma) cells stained with unpurified <u>ab108595</u> (red line). The cells were fixed with 80% methanol (5 min) and 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 (<u>ab108595</u>, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor<sup>®</sup> 488 goat anti-rabbit lgG (H&L) (<u>ab150077</u>) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) ( $0.1\mu$ g/1x10<sup>6</sup> cells) used under the same conditions. Unlabeled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab108595</u>).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labeling Lamin A + C with **ab108595** at 1/250 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab108595</u>).



Immunocytochemistry/ Immunofluorescence - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074)

Immunocytochemsitry/Immunofluorescence analysis of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling Lamin A + C with unpurified <u>ab108595</u> at 1/250 dilution. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108595**).



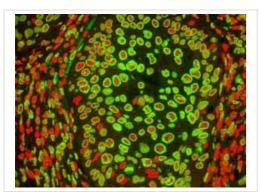
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue labeling Lamin A + C with <u>ab108595</u>.

Green - Lamin A + C.

Red - Pl.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab108595</u>).

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

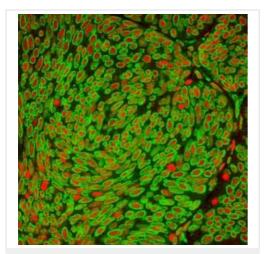


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cervical carcinoma tissue labeling Lamin A + C with <u>ab108595</u>.

Green - Lamin A + C.

Red - Pl.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108595**).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Lamin A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074)

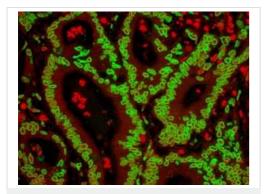
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human urothelial carcinoma tissue labeling Lamin A + C with **ab108595**.

Green - Lamin A + C.

Red - Pl.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab108595</u>).

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 A + Lamin C antibody [EPR4100] - BSA and Azide free (ab216074) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human colonic carcinoma tissue labeling Lamin A + C with <u>ab108595</u>.

Green - Lamin A + C.

Red - Pl.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab108595**).

Why choose  $\alpha$ recombinant antibody? Research with Long-term and confidence scalable supply Consistent and Recombinant reproducible results technology Success from the Ethical standards first experiment compliant Animal-free Confirmed specificity production Anti-Lamin A + Lamin C antibody [EPR4100] - BSA

and Azide free (ab216074)

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