OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-888-267-4436
Fax: +1-301-340-8606
techsupport@origene.com

OriGene Technologies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info-de@origene.com

BM4500 Monoclonal Antibody to Lamin-A/C (LMNA) - Purified

Alternate names: 70 kDa Lamin, LMN1, LMNA, Lamin A, Lamin A + C, Lamin-A/C, NY-REN-32, NYREN32, Nuclear Envelope Marker, Renal carcinoma antigen NY-REN-32

Quantity: 0.1 mg

Concentration: 1.0 mg/ml

Background: Nuclear lamins form a network of intermediate-type filaments at the nucleoplasmic site of the nuclear membrane. Two main subtypes of nuclear lamins can be distinguished, i.e. A-type lamins and B-type lamins. The A-type lamins comprise a set of three proteins arising from the same gene by alternative splicing, i.e. lamin A, lamin C and lamin Adel 10, while the B-type lamins include two proteins arising from two distinct genes, i.e. lamin B1 and lamin B2. Recent evidence has revealed that mutations in A-type lamins give rise to a range of rare but dominant genetic disorders, including Emery-Dreifuss muscular dystrophy, dilated cardiomyopathy with conduction-system disease and Dunnigan-type familial partial lipodystrophy. In addition, the expression of A-type lamins coincides with cell differentiation and as A-type lamins specifically interact with chromatin, a role in the regulation of differential gene expression has been suggested for A-type lamins.

Uniprot ID: P02545

NCBI: NP_005563.1

GeneID: 4000

Host / Isotype: Mouse / IgG3

Recommended Isotype Controls: AM03097PU-N

Clone: 133A2

Immunogen: Partially purified recombinant Human Lamin A.

Format:
State: Liquid purified Ig fraction
Buffer System: PBS
Preservatives: 0.09% Sodium Azide

Applications:
Immunoblotting: 1/100-1/1000.
Flow Cytometry:1/100-1/200.
Immunocytochemistry: 1/100-1/200.
Immunofluorescence: 1 µg/ml.
Immunohistochemistry on Frozen Sections: 1/100-1/200 with avidin-biotinylated horseradish peroxidase complex (ABC) as detection reagent.
Immunohistochemistry on Paraffin Sections: 1/2000-1/2500.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Molecular Weight: 74 kDa (Predicted)

Specificity:

The antibody 133A2 recognizes an epitope located between residues 598-611 of Lamin A, therefore it reacts exclusively with Lamin A.
Species: Human, Rat, Mouse, Bovine, Canine (Dog).
Other species not tested.

Storage:

Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Product Citations:

Purchased from Acris:
1. Mateos J, Landeira-Abia A, Fafián-Labora JA, Fernández-Pernas P, Lesende-Rodríguez I, Fernández-Puente P, et al. iTRAQ-based analysis of progerin expression reveals mitochondrial dysfunction, reactive oxygen species accumulation and altered proteostasis. Stem Cell Res Ther. 2015 Jun 12;6(1):119. doi: 10.1186/s13287-015-0110-5. PubMed PMID: 26066325.

General Readings:

1. Hozák P, Sasseville AM, Raymond Y, Cook PR. Lamin proteins form an internal nucleoskeleton as well as a peripheral lamina in human cells. J Cell Sci. 1995 Feb;108 (Pt 2):635-44. PubMed PMID: 7769007.
2. Machiels BM, Broers JL, Raymond Y, de Ley L, Kuijpers HJ, Caberg NE, et al. Abnormal A-type lamin organization in a human lung carcinoma cell line. Eur J Cell Biol. 1995 Aug;67(4):328-35. PubMed PMID: 8521872.
3. Broers JL, Machiels BM, Kuijpers HJ, Smedts F, van den Kieboom R, Raymond Y, et al. A- and B-type lamins are differentially expressed in normal human tissues. Histochem Cell Biol. 1997 Jun;107(6):505-17. PubMed PMID: 9243284.
4. Pugh GE, Coates PJ, Lane EB, Raymond Y, Quinlan RA. Distinct nuclear assembly pathways for lamins A and C lead to their increase during quiescence in Swiss 3T3 cells. J Cell Sci. 1997 Oct;110 (Pt 19):2483-93. PubMed PMID: 9410886.
5. Machiels BM, Ramaekers FC, Kuijpers HJ, Groenewoud JS, Oosterhuis JW, Looijenga LH. Nuclear lamin expression in normal testis and testicular germ cell tumours of adolescents and adults. J Pathol. 1997 Jun;182(2):197-204. PubMed PMID: 9274531.
6. Jansen MP, Machiels BM, Hopman AH, Broers JL, Bot FJ, Arends JW, et al. Comparison of A and B-type lamin expression in reactive lymph nodes and nodular sclerosing Hodgkin's disease. Histopathology. 1997 Oct;31(4):304-12. PubMed PMID: 9363444.
7. Neri LM, Raymond Y, Giordano A, Borgatti P, Marchisio M, Capitani S, et al. Spatial distribution of lamin A and B1 in the K562 cell nuclear matrix stabilized with metal ions. J Cell Biochem. 1999 Oct 1;75(1):36-45. PubMed PMID: 10462702.
8. Neri LM, Raymond Y, Giordano A, Capitani S, Martelli AM. Lamin A is part of the internal nucleoskeleton of human erythroleukemia cells. J Cell Physiol. 1999 Mar;178(3):284-95. PubMed PMID: 9989774.
9. Broers JL, Machiels BM, van Eys GJ, Kuijpers HJ, Manders EM, van Driel R, et al. Dynamics of the nuclear lamina as monitored by GFP-tagged A-type lamins. J Cell Sci. 1999 Oct;112 (Pt 20):3463-75. PubMed PMID: 10504295.
10. Broers JL, Bronnenberg NM, Kuijpers HJ, Schutte B, Hutchison CJ, Ramaekers FC. Partial cleavage of A-type lamins concurs with their total disintegration from the nuclear lamina during apoptosis. Eur J Cell Biol. 2002 Dec;81(12):677-91. PubMed

PMID: 12553668.
11. De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C, Amiel J, Boccaccio I, et al. Lamin a truncation in Hutchinson-Gilford progeria. Science. 2003 Jun 27;300(5628):2055. Epub 2003 Apr 17. PubMed PMID: 12702809.
12. Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, et al. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature. 2003 May 15;423(6937):293-8. Epub 2003 Apr 25. PubMed PMID: 12714972.

Protocols:

<u>Immunofluorescence protocol - Formaldehyde fixation</u>

1. Collect cells from T.c.unit and remove media from petri dish using suction.
2. Wash with 1x PBS and remove.
3. Incubate cells in pre-warm (37°C) Para-Formaldehyde for 12 minutes at room temperature on an orbital shaker.
4. Remove PFA and incubate in 0.5% Triton X-IOO in 1x PBS for 5 minutes at room temperature.
5. Prepare blocking reagent, this is also the antibody diluent.
6. Wash cells 2x with 1x PBS at room temperature, for 4 minutes/wash on an orbital shaker.
7. Block with 1 % NCS and 1x PBS for 30 minutes at room temperature.
8. Prepare primary antibodies (50µl/coverslip) and moist staining chambers.
9. Wash cells 2x with lx PBS at room temperature and air dry briefly.
10. Incubate with primary antibody for 1 hr at room temperature in the dark in staining chambers. During this time prepare the secondary antibody.
11. Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)
12. Incubate with secondary antibody for 1 hour at room temperature in the dark in staining chambers.
13. Wash cells 5x with 1x PBS.
14. Mount in Dapi.

Solutions (prepare fresh the same day of staining).

1x Phosphate buffered saline.
Blocking reagent: 1% NCS in 1x PBS (use fresh l0x PBS).
Fixation solution: 3.5% Para-Formaldehyde.
1.75g PFA in 20 ml d.H20 plus 5 drops 1 M NaOH. Stir on a hot plate at 50-60°C until dissolved. Add 4 drops I N HCI and check pH indicator strip. pH should be 7.4. Complete volume with d.H20 to 25ml and add 25ml 2xPBS. Check pH before adding to cover slips.

<u>Immunofluorescence protocol - Methanol/acetone fixation</u>

1. Collect cells from T.C.unit and remove media from petri dish using suction.
2. Wash with 1x PBS and remove.
3. Fix cells with cold methanol: acetone 1: 1 for 10 minutes on ice.
4. Prepare blocking reagent, this is also the diluent for the antibodies.
5. Remove fixative and wash cells 3x with Ix PBS at RT, for 4 minutes/wash on orbital shaker.
6. Block with 1% NCS and Ix PBS for 30 minutes at RT.
7. Prepare primary antibodies (50µl/coverslip) and moist staining chambers.

8. Wash cells 2x with 1 x PBS at RT and air dry for approximately 7 minutes.
9. Incubate with primary antibody for 1 hr at RT in the dark in staining chambers. During this time prepare secondary antibody.
10. Wash cells 5x with 1x PBS (5 beaker changes/5 counts in each beaker)
11. Incubate with secondary antibody for 1 hr at R T in the dark in staining chambers.
12. Wash cells 5x with 1x PBS.
13. Mount in Dapi.

Solutions (prepare fresh the same day of staining)
1x Phosphate buffered saline.
Blocking reagent: 1% NCS in 1x PBS (use fresh 10x PBS).
Fixation solution: methanol:acetone 1: 1 ice cold.

Western Blotting Protocol

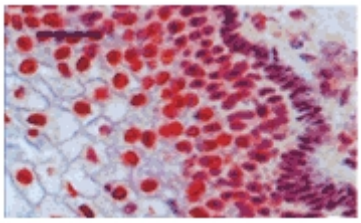
1. Transfer gel to PDVF or nitrocellulose membrane
2. Place membrane in plastic tray in blocking buffer for one hour with agitation
3. Rinse in wash buffer
4. Incubate in wash buffer plus primary antibody for one hour
5. Wash 6 X 5 minutes with wash buffer
6. Incubate in wash buffer plus secondary antibody for one hour
7. Wash 6X 5 minutes with wash buffer
8. Detect (e.g. ECL, Amersham according to manufacturers instructions)

Wash buffer
PBS + 0.1% Tween 20

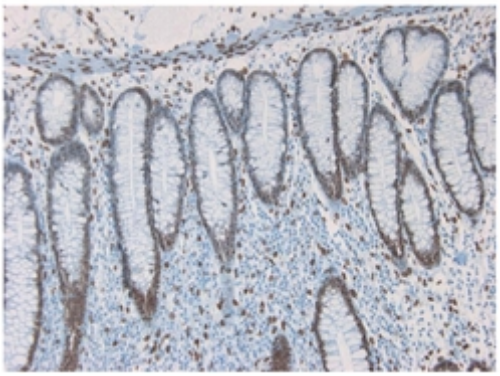
Blocking buffer
Wash buffer + 5% dried milk powder

Pictures:

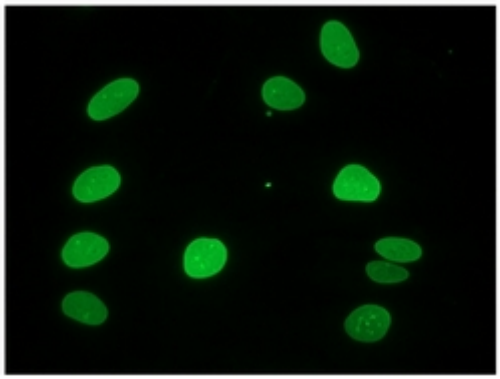
Immunostaining of human epidermis using Lamin A antibody BM4500



Immunohistochemistry on Paraffin Section of Human colon.



Immunocytochemical staining of fiboblasts showing nuclear lamina.



Immunohistochemistry on frozen sections of human colon showing nuclear lamina staining in epithelial and connective tissue cells.