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Product datasheet for BM4500

Lamin A (LMNA) Mouse Monoclonal Antibody [Clone ID: 133A2]

Product data:

Product Type:	Primary Antibodies
Clone Name:	133A2
Applications:	FC, IF, IHC, WB
Recommended Dilution:	**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.
Reactivity:	Bovine, Canine, Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG3
Clonality:	Monoclonal
Immunogen:	Partially purified recombinant Human Lamin A.
Specificity:	The antibody 133A2 recognizes an epitope located between residues 598-611 of Lamin A, therefore it reacts exclusively with Lamin A.
Formulation:	PBS State: Purified State: Liquid purified Ig fraction Preservative: 0.09% Sodium Azide
Concentration:	lot specific
Conjugation:	Unconjugated
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.
Stability:	Shelf life: one year from despatch.
Predicted Protein Size:	74 kDa (Predicted)
Gene Name:	lamin A/C

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Database Link: Entrez Gene 4000 Human
P02545

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.

Synonyms: LMNA, LMN1, 70 kDa Lamin, NY-REN-32, NYREN32, Lamin-A/C, Lamin A, Lamin A + C, Nuclear Envelope Marker

Note: Protocol: **Immunofluorescence protocol - Formaldehyde fixation**
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.

Immunofluorescence protocol - Methanol/acetone fixation

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.

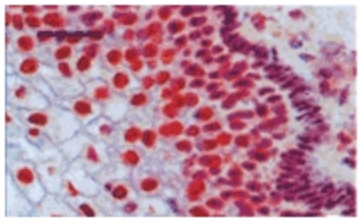
Western Blotting Protocol

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)

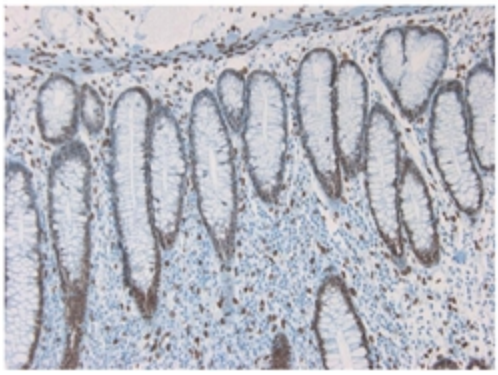
Wash buffer
PBS + 0.1% Tween 20

Blocking buffer
Wash buffer + 5% dried milk powder

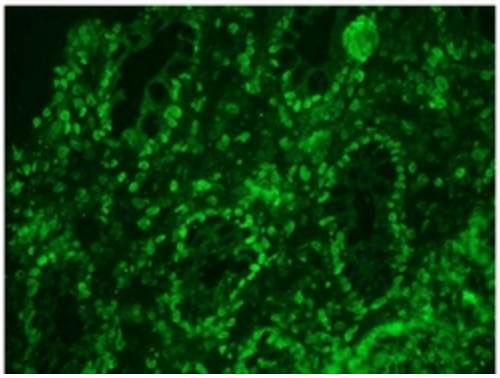
Product images:



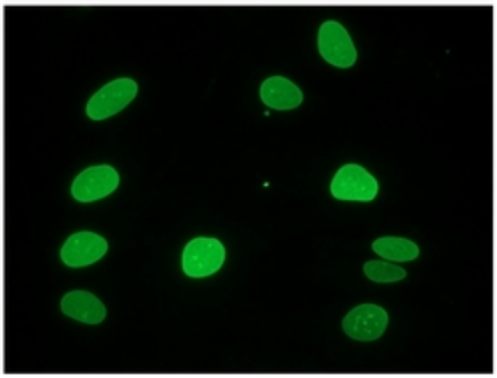
Immunostaining of human epidermis using Lamin A antibody



Immunohistochemistry on Paraffin Section of Human colon.



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



Immunocytochemical staining of fiboblasts showing nuclear lamina.