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 EU

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Monoclonal Antibody to Lamin-A/C (LMNA) - Supernatant

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

Catalog No.: BM4505S

Quantity: 1 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 / IgG2

Clone: JOL5

Immunogen: Recombinant bacterially expressed human lamin A.

Format: **State:** Tissue Culture Supernatant with 0.09% Sodium Azide as preservative.
Purification: Unpurified.

Applications: **Immunofluorescence:** 1/10 (as a guideline).
Western blot (ECL technique): 1/50.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Detects an un-phosphorylated form of lamin A/C and the epitope is mapped to the N-terminal head domain of both proteins.
Species: Human and mouse.
Other species not tested.

Storage: Store the antibody 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.

For research and in vitro use only. Not for diagnostic or therapeutic work.
Material Safety Datasheets are available at www.acris-antibodies.com or on request.

Acris Antibodies is now part of the OriGene family. Learn more at www.origene.com

General Readings:

1. Dyer JA, Kill IR, Pugh G, Quinlan RA, Lane EB, Hutchison CJ. Cell cycle changes in A-type lamin associations detected in human dermal fibroblasts using monoclonal antibodies. Chromosome Res. 1997 Sep;5(6):383-94. PubMed PMID: 9364940.

Protocols:

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 1M NaOH. Stir on a hot plate at 50-60°C until dissolved. Add 4 drops IN 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.

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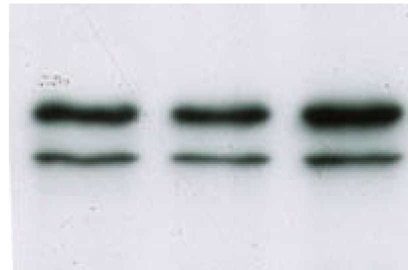
Western Blotting Protocol
1. Transfer gel to PDVF or nitrocellulose membrane
2.Place membrane in plastic tray in blocking buffer for one hour with agitation
3. Rinse in wash buffer
4. Incubate in wash buffer plus primary antibody for one hour
5. Wash 6 X 5 minutes with wash buffer
6. Incubate in wash 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.

The concentration of antibodies used depends on each antibody, the amount of antigen and the detection method used. Generally, dilution is in the range of a few hundred times dilution to a few thousand times dilution, but usually has to be determined empirically

Pictures:

Figure 1. Western blot using Lamin A/C (JoL5) showing extracts from mouse myoblasts c2c12. Upper band is lamin A and the lower is lamin C.



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