For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-Nucleolin mAb

Code No.	Clone	Subclass	Quantity	Concentration
M019-3	4E2	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: Nucleolin is a major nucleolar phosphoprotein which is associated with preribosomal RNA and is implicated in the early stage of preribosomal RNP assembly and processing. This 100 kDa protein can be subdivided into three major domains: The N-terminal domain comprises long acidic stretches interspersed with basic repeats, similar to the structure of a high mobility group-type protein. This domain is responsible for the ability of nucleolin to modulate chromatin condensation. In contrast, the central domain contains four RNA binding elements. Finally, the C-terminal domain, approximately 85 amino acids long, is strikingly rich in glycine, arginine, and phenylalanine residues. Nucleolin fluctuates in parallel to DNA synthesis. The intact 100 kDa nucleolin molecule is the major species in actively dividing cells, whereas the degraded forms are relatively abundant in nondividing cells. Stability of nucleolin molecule is cell proliferation-dependent. Nucleolin is found to be identical to the human DNA helicase IV. Nucleolin/DNA helicase IV can unwind RNA-RNA duplexes, as well as DNA-DNA and DNA-RNA duplexes. Furthermore, nucleolin directly interacts with DNA topoisomerase I.

SOURCE: This antibody was purified from mouse ascites fluid using protein A agarose. This hybridoma (clone 4E2) was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with human nucleolin from Raji cell extract.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human nucleolin on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL for chemiluminescence detection system
Immunoprecipitation; Not tested*
*It is reported that this antibody can be used in this application in the reference number 2), 6) and 11).
Immunohistochemistry; 1-10 µg/mL
Immunocytochemistry; 1-10 µg/mL
Flow cytometry; 5-10 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOLS**.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Monkey*	Bovine†	Dog§
Cells	HL-60, ZR-75-1, Jurkat, Raji, HeLa	WR19L	Rat-1	Not tested	Not tested	Not tested
Reactivity on WB	+	-	-			

*It is reported in the reference number *9), †6) and §10).

INTENDED USE:
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REFERENCES:

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3) Sakaguchi, M., *et al., PNAS*. **102**, 13921-13926 (2005) [WB]
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5) Sorokina, E. A., *et al., J. Am. Soc. Nephrol.* **15**, 2057-2065 (2004) [WB, IC]
6) Baran, V., *et al., Eur. J. Immunol.* **33**, 2557-2566 (2003) [WB, IP]
7) Barel, M., *et al., Biol Reprod.* **70**, 877-886 (2004) [IC]
8) Gueven, N., *et al., Hum. Mol. Genet.* **13**, 1081-1093 (2004) [WB, IC]
9) Guo, Y. X., *et al., Virology* **306**, 225-235 (2003) [IC]
10) Kumar, V., *et al., J. Am. Soc. Nephrol.* **14**, 289-297 (2003)
11) Morimoto, H., *et al., J. Histochem. Cytochem.* **50**, 1187-1193 (2002) [WB, IP, IC]

As clone 4E2 is really famous all over the world, a lot of researches have been reported. These references are a part of such reports.

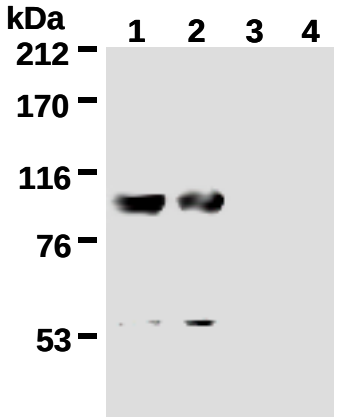
PROTOCOLS:

SDS-PAGE & Western Blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.

4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of sample per lane on a 1-mm-thick SDS-polyacrylamide gel and carry out electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacturer's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggested in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Western blotting; Jurkat and HeLa)

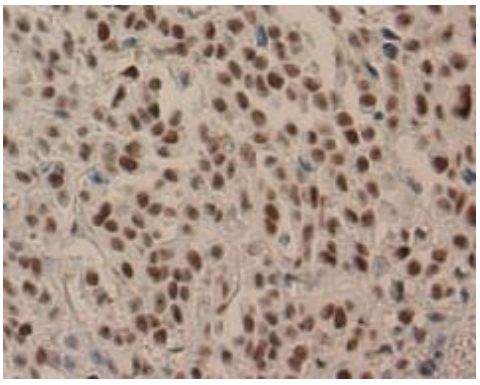


Western blot analysis of Nucleolin expression in Jurkat (1), HeLa (2), WR19L (3) and Rat-1 (4) using M019-3.

Immunohistochemical staining for paraffin-embedded sections: SAB method

1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Remove the slides from PBS and cover each section with 3% H_2O_2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent (Ultratech HRP Kit; MBL, code no. IM-2391) for 5 minutes to block non-specific staining. Do not wash.
6) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggested in the **APPLICATIONS**.
7) Incubate the sections for 1 hour at room temperature.
8) Wash the slides 3 times in PBS for 5 minutes each.
9) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
10) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 10 minutes at room temperature. Wash as in step 8).
11) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
12) Wash the slides in water for 5 minutes.
13) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
14) Now ready for mounting.

(Positive control for Immunohistochemistry; Human stomach)



Immunohistochemical detection of Nucleolin on paraffin embedded section of human stomach with M019-3.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1x10^4 cells for one slide, then incubate in a CO_2 incubator for one night.)
2) Wash the cells 3 times with PBS.
3) Fix the cells by immersing the slide in PBS containing 4% paraformaldehyde for 20 minutes at room temperature.

4) The glass slide was washed with PBS 3 times.
5) Immerse the slide in PBS containing 0.1% TritonX-100 for 10 minutes at room temperature.
6) The glass slide was washed 3 times with PBS.
7) Add the primary antibody diluted with PBS as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
8) The glass slide was washed 3 times with PBS.
9) Add FITC conjugated anti-mouse IgG antibody diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
10) The glass slide was washed 3 times with PBS.
11) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
12) Promptly add mounting medium onto the slide, then put a cover slip on it.

Flow cytometric analysis for cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3].
   *Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
2) Add 200 μL of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4°C.
3) Wash the cells 3 times with washing buffer.
4) Add 200 μL of 70% ethanol to the cell pellet after tapping. Mix well, then permeablize the cells for 30 minutes at -20°C.
5) Wash the cells 3 times with washing buffer.
6) Add 20 μL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
7) Add 30 μL of the primary antibody (4E2) as suggested in the **APPLICATIONS** diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add FITC conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
10) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
11) Resuspend the cells with 500 μL of the washing buffer and analyze by a flow cytometer.

RELATED PRODUCTS:

M019-3S	Anti-Nucleolin mAb (4E2)
RN090PW	Anti-DDX21 pAb
RN101PW	Anti-FBL (Fibrillarin) pAb
M075-3	Mouse IgG1 (isotype control) (2E12)