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Product datasheet for AM26580AF-N

Nucleolin (NCL) Mouse Monoclonal Antibody [Clone ID: 4E2]

Product data:

Product Type:	Primary Antibodies
Clone Name:	4E2
Applications:	FC, IF, IHC, WB
Recommended Dilution:	**Western blot:** 1 µg/ml for chemiluminescence detection system. **Immunohistochemistry (paraffin sections):** 1-10 µg/ml. **Immunocytochemistry:** 1-10 µg/ml. **Flow cytometry:** 5-10 µg/ml (final concenctration).
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Human nucleolin from Raji cell extract
Specificity:	This antibody detects nucleolin.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservatives are contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Upon receipt, store undiluted (in aliquots) at -20°C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	nucleolin
Database Link:	Entrez Gene 4691 Human P19338
Background:	Nucleolin (NCL), a eukaryotic nucleolar phosphoprotein, is involved in the synthesis and maturation of ribosomes. It is located mainly in dense fibrillar regions of the nucleolus. Human NCL gene consists of 14 exons with 13 introns and spans approximately 11kb. The intron 11 of the NCL gene encodes a small nucleolar RNA, termed U20.

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Synonyms: NCL

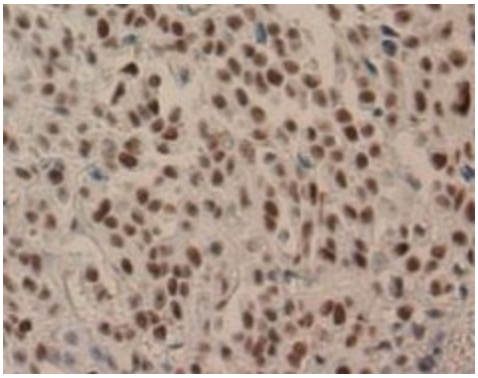
Note: This product was originally produced by MBL International.

Protocol:
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 o 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 o 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 µ L 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, 2 0% MeOH). See the manufacture r '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 o C.
7) Incubate the membrane with primary antibody d iluted with PBS, pH 7.2 containing 1% skimmed milk as suggest ed 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) I ncubate the membrane with the 1:1 0, 000 HRP - conjugated anti - mouse IgG 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. Th e condition for exposure and development may vary.
(Positive controls for Western blotting; Jurkat, HeLa)
Immunohistochemical staining for paraffin - embedded sections : SAB method
1) Deparaffinize the sections with Xylene 3 times for 3 - 5 minutes each.
2) Wash th e 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 2 O 2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 tim es in PBS for 5 minutes each.
5) Remove the slides from PBS, wipe gently around each section and cover tissues with

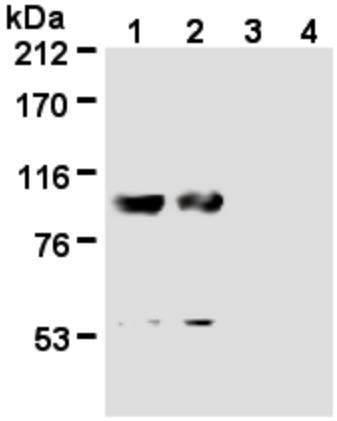
Protein Blocking Agent for 5 minutes to block non - specific staining. Do not wash.
6) Tip off the blocki ng buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest ed in the APPLICATIONS .
7) Incubate the section s for 1 hour at room temperature.
8) Wash the slide s 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 1 0 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 µ L of 30% H 2 O 2 in 150 m L 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 i mmerse 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)
Immunocytochemistry
1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 1x 10e4 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 cont aining 4% paraformaldeh y de for 20 minutes at roomtem perature.
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 primar y antibody diluted with PBS as suggest ed in the APPLICATIONS onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.
8) The glass slide was washed 3 times w ith PBS.
9) Add 10 0 µ L of 1: 100 FITC conjugated anti - mouse IgG diluted with PBS onto the cells. Incubate for 30 mi n utes at room temperature. Keep out light by aluminum foil.
10) The glass slide was washed 3 times with PBS.
11) Wipe excess liqu id 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 step s described below.
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN 3].
2) Add 200 µ L of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4 o C .

3) Wash the cells 3 times with washing buffer.
4) Add 200 µ L of 70% ethanol to the cell pellet after tapping. Mix well, then permeablize the cel ls for 30 minutes at - 20 o C.
5) Wash the cells 3 times with washing buffer.
6) Add 2 0 µ L of Clear Back (human Fc receptor blocking reagent) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
7) Add 30 µ L of the primary antibody (4E2) as suggest ed in the APPLICATIONS diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
8) Add 1 m L of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature . Remove supernatant by careful aspiration.
9) Add 30 µ L of 1:100 FITC conjugated anti - mouse IgG diluted with the washing buffer . Mix well and incubate for 15 minutes at room temperature.
10) Add 1 m L 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 µ L of the washing buffer and analyze by a flow cytometer.

Product images:



Immunohistochemical detection of Nucleolin on paraffin embedded section of human stomach with AM26580AF-N.



Western blot analysis of Nucleolin expression in Jurkat (1), HeLa (2), WR19L (3) and Rat-1 (4) using AM26580AF-N.