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Product datasheet for AM26657AF-N

PCNA Mouse Monoclonal Antibody [Clone ID: 5A10]

Product data:

Product Type:	Primary Antibodies
Clone Name:	5A10
Applications:	FC, IHC, WB
Recommended Dilution:	Western blot: 5 µg/ml: Flow cytometry: 10 ~ 50 µg/ml. Immunohistochemistry on paraffin sections: 2 µg/ml; Microwave treatment will be recommended; Treat with Citrate Buffer (pH 6.0) for 10 ~ 20 minutes. For details see protocols below.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Recombinant rat PCNA
Specificity:	This antibody reacts with PCNA.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein-A Sepharose
Conjugation:	Unconjugated
Storage:	Upon receipt, store (in aliqouts) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: One year from despatch.
Gene Name:	proliferating cell nuclear antigen
Database Link:	Entrez Gene 5111 Human P12004
Synonyms:	Cyclin
Note:	This product was originally produced by MBL International.

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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 oC 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 oC and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the 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 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system. (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture'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 oC.
7) Incubate the membrane with primary antibody diluted as suggested in the APPLICATIONS for 1 hour at room temperature. (The optimal concentration of antibody will depend on the experimental conditions.)
8) Wash the membrane with PBS (5 minutes x 6 times).
9) Incubate the membrane with the 1:10000 POD-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 (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
12) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

Immunohistochemical staining For tissue section: ABC method
1) Deparaffinize section, hydrate to water.
*Microwave treatment will be recommended
2) Wash in PBS for 5 minutes before starting the staining.
3) Remove slides from PBS and cover each section with 100~200 µL of 3% H2O2 for 10 minutes to block endogenous peroxidase activity. Do not treat frozen section with 3% H2O2. Wash in PBS for 2 minutes.
4) Remove slides from PBS, wipe gently around each section and cover tissues with 100~200 µL of blocking buffer (1% Normal Goat Serum in PBS) for 10 minutes to block non specific antibody staining. Do not wash.
5) Tip off the blocking buffer, wipe gently around each section and cover tissues with 100~200 µL of primary antibody at the concentration suggested in APPLICATIONS (The optimal concentration of antibody will depend on the experimental conditions and the abundance of

the antigen) in Protein Blocking Agent.
6) Incubate the section for 1 hour at room temperature (37°C for 10 minutes).
7) Gently wash the slide with a stream of buffer from a wash bottle or pipettor: do not touch the tissue section with the wash bottle or pipet tip. Wash 3 times with PBS for 5 minutes each.
8) Wipe gently around each section and cover tissues with 100~200 µL of biotinylated goat anti-mouse IgG (H+L)(Fab')2.
9) Incubate for 10 minutes at room temperature.
10) Wash as in 7).
11) Wipe gently around section and cover tissues with 100~200 µL of Streptavidin conjugated HRP.
12) Incubate for 10 minutes at room temperature.
13) Wash as in 7).
14) Visualize with DAB substrate/chromogen (25 mg of DAB in 100 mL of PBS plus 2~3 mL of 0.3% H2O2) for approximately 1~8 minutes. Wash in distilled water.
*DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
15) Counterstain in hematoxylin.
16) Mounting.

Flow cytometric staining
We recommend you to use fisher tube or equivalent as reaction tube for all step described below.
1) Harvest and count cells. (5 x 10e5 cells/tube)
2) Wash the cells. (Suspend in wash buffer (PBS/0.1% NaN3/2% FCS/pH 7.4) and centrifuge at 400 x g for 1 minute and discard the supernatant).
3) Aspirate and wash the cells.
4) Add 200 µL of 70% methanol with mixing by vortex and incubate for 30 minutes at -20°C.
5) Add 1 mL of wash buffer and centrifuge at 400 x g for 10 minutes. Discard the supernatant.
6) Add 10µL of Normal Goat Serum and incubate for 20 minutes at room temperature.
7) Add 30 µL of primary antibody diluted as suggested in APPLICATIONS (The optimal concentration of antibody will depend on the experimental conditions) to cell pellet and gently mix then incubate for 30 minutes at room temperature.
8) Add 1mL of wash buffer and centrifuge at 400 x g for 1 minute.
9) Aspirate supernatant.
10) Add 30µL of FITC-conjugated anti-mouse IgG diluted 1:100 in wash buffer and incubate for 15 minutes at room temperature.
11) Add 1 mL of wash buffer and centrifuge at 400 x g for 5 minutes.
12) Aspirate supernatant.
13) Resuspend the cell pellet in 0.5 mL of wash buffer and analyze stained cells within 4 hours.