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MBL
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MONOCLONAL ANTIBODY

Anti-PCNA mAb

Code No.	Clone	Subclass	Quantity	Concentration
MH-12-3	5A10	Mouse IgG1	100 µg	1 mg/mL

BACKGROUND: The proliferating cell nuclear antigen (PCNA) also known as cyclin, that appearance correlates with the proliferative state of the cells. PCNA level increases 2 to 3 times between early G_1 and S phases, it shows maximum being during S phase and declines during G_2 and M phases. It is revealed to be a co-factor of DNA polymerase δ and to be necessary for DNA synthesis and cell cycle progression. Examination of PCNA is important in the malignant tumor research.

SOURCE: This antibody was purified from hybridoma (clone 5A10) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3U1 with Balb/c mouse splenocyte immunized with recombinant rat PCNA.

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 -20ºC.

REACTIVITY: This antibody reacts with human, mouse and rat PCNA on Western blotting, Immunoprecipitation, Flow cytometry, Immunohistochemistry and Immunocytochemistry.

APPLICATIONS:

Western blotting; 5 µg/mL for chemiluminescence detection system
Immunoprecipitation; 5 µg/200 µL of cell extract from 2.5 x 10^6 cells
Immunohistochemistry; 2 µg/mL
Heat treatment is necessary for paraffin embedded sections.
Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.5)
Immunocytochemistry; 10 µg/mL
Flow cytometry; 10-50 µg/mL (final concentration)

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Raji, Hep-2	WR19L	PC12
Reactivity on WB	+	+	+

REFERENCES:

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2) Haraguchi, T., *et al., J. Cell Sci*. **120**, 1967-1977 (2007)
3) Kudoh, A., *et al*., *J. Virol*. **78**, 104-115 (2004)
4) Shiomi, Y., *et al., Genes Cells* **9**, 279-290 (2004)
5) Fujita, K., *et al., Genes Cells* **8**, 559-571 (2003)
6) Suga, S., *et al., J. Am. Soc. Nephrol*. **14**, 397-406 (2003)
7) Iida, T., *et al., Genes Cells* **7**, 997-1007 (2002)
8) Ibe, S., *et al., Genes Cells* **6**, 815-824 (2001)
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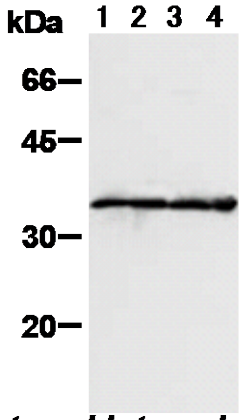
10) Miyachi, K., *et al., J. Immunol*. **121**, 2228-2234 (1978)
11) Prelich, G., *et al., Nature* **326**, 517-520 (1987)
12) Matsumoto, K., *et al., EMBO J*. **6**, 637-642 (1987)

Clone 5A10 is used in reference number 1) - 9).

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



Western blot analysis of PCNA expression in Raji (1), Hep-2 (2), WR19L (3) and PC12 (4) using MH-12-3.

PROTOCOLS:

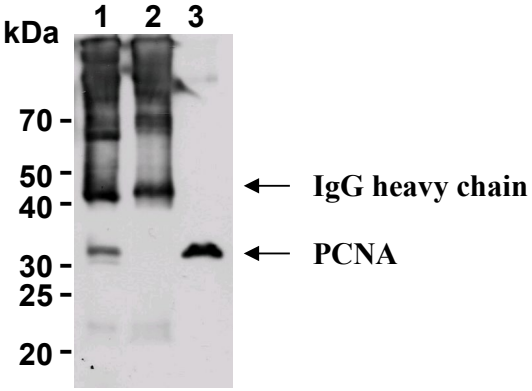
SDS-PAGE & Western Blotting

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 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/cm^2 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°C.
7) Incubate the membrane with primary antibody diluted as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (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 (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.

(Positive controls for Western blotting; Raji, Hep-2, WR19L, PC12)

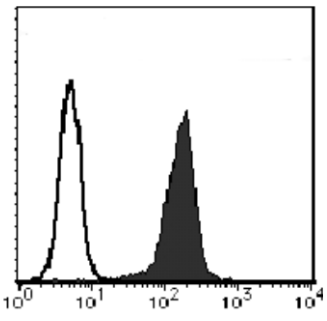


Immunoprecipitation of PCNA from Raji with MH-12-3 (1) or mouse IgG1 (2) *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with MH-12-3. Raji crude lysate was resolved in lane 3.*

Immunoprecipitation

1) Collect the cultured cells from 75-cm^2 flask (containing about 1 x 10^7 cells).
2) Wash the cells 2 times with PBS and suspend with 800 µL of cold Lysis buffer (50 mM HEPES-KOH, pH 7.5, 250 mM NaCl, 0.1% NP-40, 5 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).
3) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
4) Add 50 µL of 50% protein G agarose beads in the supernatant. Incubate it at 4°C with rotating for 60 minutes.
5) Centrifuge the tube at 12,000 x g for 5 minutes at 4°C. Supernatant is equally divided into another two tube.
6) Add the mouse IgG1 isotype control antibody (MBL; code no. M075-3) or anti-PCNA antibody at the amount of as suggested in the **APPLICATIONS** to the supernatant. Vortex briefly and incubate with gently agitation for 30-120 minutes at 4°C.
7) Add 20 µL of 50% protein G agarose beads into the tube. Mix well and incubate with gentle agitation for 30-60 minutes at 4°C.
8) Wash the beads 3-5 times with ice-cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
9) Resuspend the beads in 30 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes. Use 15 µL/lane for the SDS-PAGE analysis.
(See **SDS-PAGE & Western blotting**.)

(Positive control for Immunoprecipitation; Raji)



Flow cytometric analysis of PCNA expression on Raji cells. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of MH-12-3 to the cells.*

Flow cytometric analysis for floating cells

We recommend you to use fisher tube or equivalent as reaction tube for all steps 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 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 permeabilize the cells for 30 minutes at -20ºC.
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 (20~25ºC).
7) Add 40 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in 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 40 µL of 1:160 FITC conjugated anti-mouse IgG (MBL; code no. 238) 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.
(Positive control for Flow cytometry; Raji)

Immunohistochemical staining for paraffin-embedded sections

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) Heat treatment
Heat treatment by Microwave:
Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.
5) Remove the slides from the citrate buffer and cover each section with 3% H_2O_2 in PBS for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) 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.
7) 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**.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
12) 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.
13) Wash the slides in water for 5 minutes.
14) 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.
15) Now ready for mounting.

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (for example, spread 10^4 of cells per one well, then incubate in a CO_2 incubator for one night.)
2) Fix the cells by immersing the slide in Acetone for 10 minutes on ice.
3) Air dry the slides.
4) Add the 40 µL of primary antibody diluted with PBS as suggest in the **APPLICATIONS** onto the cells and incubate for 1 hour at room temperature. (Optimization of antibody concentration or incubation condition are recommended if necessary.)
5) Prepare a wash container such as a 500 mL beaker with a magnetic stirrer. Then wash the cultured cells on the glass slide by soaking the slide with a plenty of PBS in the wash container for 5 minutes. Take care not to touch the cells. Repeat washes once more.
6) Add 40 µL of 1:100 FITC conjugated anti-mouse IgG (MBL; code no. IM-0819) diluted with PBS onto the cells. Incubate for 30 minutes at room temperature. Keep out light by aluminum foil.
7) Wash the slide in a plenty of PBS as in the step 5).
8) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
9) Promptly add mounting medium onto the slide, then put a cover slip on it.

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