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MOUSE ANTI-PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB424R (Formerly Roche Cat. #1742353)

LOT NUMBER:

QUANTITY: 100 µg

CONCENTRATION: 1 mg/mL

SPECIFICITY: Proliferating cell nuclear antigen (PCNA) was first described by E. M. Tan *et al.* as the antigen for a subpopulation of autoantibodies in patients with systemic lupus erythematosus (l-3). It was found later to be identical to cyclin (4) and the auxiliary protein of DNA polymerase-δ (5,6). The DNA-sequence of human PCNA has been determined (7). PCNA begins to accumulate during the G1 phase of the cell cycle, is most abundant during the S phase, and declines during the G2/M phase (8). The temporal specificity of PCNA makes it an ideal marker for cell proliferation. Anti-proliferating cell nuclear antigen has been used to identify transformed cells (8) proliferating cells in solid tumors (9) and blast cells in leukemia patients (10). Immunohisto-chemistry with clone PC10 has been used to study the expression of PCNA in paraffin sections of normal tissues and lymphoid neoplasms (11).

IMMUNOGEN: Rat PCNA produced in the protein A expression vector pR1T2T (12).

ISOTYPE: IgG_{2a}

CLONE NAME: PC10

APPLICATIONS:
Western blot: 0.1-1.0 µg/ml
Flow Cytometery: 1:100
Immunohistochemistry: (Formalin fixed paraffin embedded tissues) :
5-50 µg/ml
Optimal working dilutions must be determined by end user.

SPECIES REACTIVITIES: Human, mouse, insect cells.

FORMAT: Purified immunoglobulin (ion-exchange chromatography)

PRESENTATION: Liquid.

STORAGE/HANDLING: Maintain at 2-8°C in undiluted aliquots for up to 6 months after date of receipt.

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APPLICATION PROTOCOLS

Immunohistochemistry

The working concentration for immunohistochemistry depends on the length of time slides are incubated with anti-PCNA If an overnight incubation at +4°C is desired, use a 1:200 dilution of anti-proliferating cell nuclear antigen for an antibody concentration of 5 μg/ml in PBS containing 1% normal serum. If a one-hour incubation at room temperature is desired, use a 1:20 dilution of anti-proliferating cell nuclear antigen for an antibody concentration of 50 ug/ml in PBS containing 1% normal serum.
The procedure below was developed to localize PCNA in formalin-fixed, paraffin-embedded human tonsil tissue. Perform all steps at room temperature unless otherwise indicated. Where normal serum is indicated, use normal serum from the same species as the source of the secondary antibody. We recommend that you use a humidified incubation chamber. This procedure represents suggested guidelines for the use of anti-PCNA. Antibody concentrations and incubation conditions for a given experimental system should be determined empirically.

Prepare slides and block endogenous *peroxidase*

1. Cut 5 pm sections of the formalin-fixed, paraffin-embedded tissue and place each section onto a poly-L-lysine-coated slide.
2 . Immerse the slides in three changes of xylene, two minutes per immersion.
3 . Immerse the slides in three changes of absolute alcohol, two minutes per immersion.
4. Incubate the slides in absolute methanol containing 1.2% H_2O_2, for 15 minutes.
5. Wash the slides in deionized water for 2 minutes.
6. Store the slides in PBS until you are ready to use them.

Label with anti-PCNA

1. Incubate the slides in phosphate-buffered saline containing 1% normal serum (PBS-NS) for 15 minutes. This step blocks non-specific binding.
2. The slides may be incubated with anti-PCNA in one of two ways:

a. Apply 100 μl anti-PCNA (diluted in PBS-NS to a final concentration of 50 μl/ml) to each slide and incubate for 60 minutes at room temperature.

b. Apply 100-200 μl anti-PCNA (diluted in PBS-NS to a final concentration of 5 μg/ml) to each slide and incubate overnight at +4°C.

3. Wash the slides three times in PBS.
4 . Detect with a standard secondary antibody detection system.

Flow cytometry

Depending on the fixation method employed, monoclonal antibody PC10 can be used for flow cytometric detection of cells in the S-phase of the mitotic cycle (fixation method A,.below), or all proliferating cells (fixation method B; reference 13). In either case, the antibody should be titrated to determine the best concentration for your system.

Fixation method A

(i). Incubate l-2 x I0^6 cells in 1 ml of prechilled lysis buffer (PBS containing 0.5% Triton X-100, 0.2 μg/ml EDTA,and 1% bovine serum albumin) for 15 minutes.
(ii). Gradually add 3 ml of cold 100% methanol. Incubate for 10 minutes at -20°C.
(iii). Wash the cells with PBS, centrifuging at 200 x g for IO minutes.

Fixation method B

(i). Incubate l-2 x 1 0^6 cells in 100% methanol for 30 minutes at -20°C.
(ii). Wash the cells in pre-chilled PBS containing 0.5% Nonidet P40, centrifuging at 200 x g for 10 minutes.
(iii). Wash the cells with PBS, centrifuging at 200 x g for 10 minutes.

Preparation for flow cytometry

1. Resuspend the cells in 100 μl of PBS containing 1 μg of anti-PCNA clone PC-IO (I:100 dilution). Incubate for 30 minutes.
2. Wash the cells twice with PBS, centrifuging at 200 x g for 10 minutes in each wash.
3 . Incubate the cells in 100 μlof a I:20 dilution of fluorescein-conjugated goat anti-mouse IgG.
4. Wash the cells twice with PBS, centrifuging at 200 x g for 10 minutes.in each wash.

Western blotting

Anti-PCNA clone PC1 0 has been used to detect PCNA in Western blot analysis of extracts from MOLT-4 cells, insect cells, and *Schizosaccharomyces* pombe (12). The following procedure can be used for cultured mammalian cells (e.g., mouse fibroblasts) in mid-log phase. Antibody concentra-tions should be determined empirically.
1. Harvest, wash, and resuspend cells in reducing sample buffer for SDS-PAGE. A final protein concentration of 2-4 μg/ml is recommended.
2. Sonicate the samples and incubate them in a 100°C water bath for one minute.
3. Load 20-40 μg of protein per lane onto a 3-l7% SDS-PAGE gradient gel.
4. Perform electrophoresis and transfer the protein to a nitrocellulose membrane.
5. Incubate the membrane with anti-PCNA for 30 minutes at +37°C.
6. Wash the membrane for 5 minutes in PBS containing 0.01% Triton X-l 00. Wash the membrane twice, 5 minutes per wash, in PBS.
7. Incubate the membrane with alkaline-phosphatase-conjugated goat anti-mouse IgG (diluted I:500 in PBS).
8. Wash the membrane for 5 minutes in PBS containing
0.01% Triton X-l 00. Wash the membrane twice, 5 minutes per wash, in PBS.
9. Incubate the membrane in PBS containing equal amounts of 5-Bromo-4-chloroS-indolyl-phosphate.

PCNA should appear as a 36 kD band.

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