Product No. F-0167
Lot 047H4827

Monoclonal Anti-Proliferating Cell Nuclear Antigen FITC Conjugate

Purified Mouse Immunoglobulin
Clone PC10

FITC Conjugated Monoclonal Anti-Proliferating Cell Nuclear Antigen (PCNA) is a purified mouse monoclonal antibody conjugated with fluorescein isothiocyanate (FITC) isomer I. Monoclonal Anti-Proliferating Cell Nuclear Antigen (mouse IgG2a isotype) is derived from the PC10 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from immunized BALB/c mice. Rat PCNA-Protein A fusion protein was used as immunogen.[1] The isotype is determined using Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The conjugate is provided as a solution (300 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

Specificity

FITC Monoclonal Anti-Proliferating Cell Nuclear Antigen (PCNA) recognizes the acidic non-histone auxiliary protein for DNA polymerase δ, PCNA (36 kD), also known as polymerase delta accessory protein. Cross reactivity has been observed with vertebrates (human, monkey), insects, and yeasts.[1] The conjugated antibody may be used in flow cytometric multiparameter cell cycle analysis. Identification of S-phase by flow cytometry using this antibody requires special fixation procedures.[9-13]

Description

Proliferating cell nuclear antigen (PCNA, 36 kD), also known as cyclin, is an auxiliary protein of DNA polymerase δ, that is essential for DNA replication at S-phase. The protein is present in nucleoplasm of continually cycling cells throughout the cell cycle.[1-8] PCNA begins to accumulate during the G_1 phase of the cell cycle, is most abundant during the S phase, and declines during the G_2/M phase. The predominant distribution of PCNA appears to change with the stage of the cell cycle. In early S phase, PCNA has a very granular distribution and is absent from the nucleoli, while at late S phase, prominent presence in the nucleoli is evident. Cells which have left the cell cycle to enter the G_0 phase may still express PCNA due to its long half life (>20 hours) relative to the rate of cell turnover. PCNA is a conserved protein as can be seen by the amino acid sequence homology among mammalian PCNAs.[1] Experiments using anti-sense oligonucleotides and micro-injection of antibodies strongly suggest that PCNA is essential for cellular DNA synthesis. It is required for leading strand synthesis in the SV40 system where it probably acts as an auxiliary protein for polymerase δ, coordinating leading and lagging strand synthesis and rendering the polymerase more processive. In many normal tissues, PCNA-positive cells are limited to the proliferative compartment. In many tumors the proportion of PCNA-positive cells exceeds that expected for the proportion of proliferating cells, as assessed by other methods. In addition, apparently normal epithelium adjacent to tumors in breast tissue, liver and pancreas or to inflammatory gastric or hepatic lesions has been shown to overexpress PCNA. It has been postulated that this increased expression of PCNA in tumors is due to growth factors that upregulate the concentration of this protein. Measurements of cell turnover and proliferation are employed in a wide variety of clinical and experimental investigations of cell and tissue kinetics. In clinical medicine, such studies performed in combination with other complementary assessments are providing new and important information that enables finer discrimination in tumor diagnosis. Determination of the steady-state or growth phase of tumors contributes an influential diagnostic tool for the design of appropriate therapeutic treatment.[8] Several studies have demonstrated correlations between measurements of PCNA immunoreactivity and known prognostic variables in a range of malignant neoplasms.[4] PCNA immunohistochemistry[1-8] is a potentially valuable tool for defining proliferative activity in diagnostic pathology because it can be performed on formalin-fixed and processed tissues and it is independent of exogenous tracer molecules such as tritiated thymidine or bromodeoxyuridine.[6]

Uses

FITC Monoclonal Anti-Proliferating Cell Nuclear Antigen (PCNA) may be used for the localization of PCNA by flow cytometry.

F/P Molar Ratio: 7.4

Performance

When assayed by flow cytometric analysis using 10 µl of the antibody to stain 1 x 10^6 cells the percent population positive is similar to that obtained with saturating monoclonal antibody levels.

In order to obtain best results, it is recommended that each

user determine the optimal working dilution for individual applications by titration assay.

Storage

Store at 2-8°C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

* Due to the sodium azide content a material safety sheet (MSDS) has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Procedure for Direct Immunofluorescent Staining

Reagents and Materials Needed but Not Supplied

1. Human cell suspension (e.g., TF1 cultured cell line (erythroleukemia).
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Sigma Product No. F-6522).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. 25% Normal goat serum in diluent
7. Centrifuge.
8. Counting chamber.
9. Trypan blue (Sigma Product No. T-0776), 0.2% in 0.01 M PBS, pH 7.4.
10. 2% paraformaldehyde in PBS.
11. Acetone
12. Methanol
13. Flow cytometer.

Procedure

1. Adjust cell suspension to 1 x 10^7 cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g., trypan blue).
2. Pellet cells by centrifugation at 500 x g for 5 minutes at room temperature.
3. Remove supernatant by careful aspiration.
4. Resuspend cells in 20 ml 100% acetone at −20°C for 15 minutes.
5. Repeat steps 2 and 3.
6. Resuspend cells in 20 ml 100% methanol at −20°C for 15 minutes. Disperse cell clumps by repeated drawing of the suspension through a pipet.
7. Repeat steps 2 and 3.
8. Resuspend cells in 5 ml 25% normal goat serum in diluent for 30 minutes at room temperature.
9. Repeat steps 2 and 3.
10. Resuspend cells in initial volume of diluent. For each sample, add 100 µl or 1 x 10^6 cells per tube.
11. Add 10 µl of conjugate to tube(s) containing cells to be stained. Vortex tube gently. rotect from light/ Incubate the cells at room temperature (18 - 22°C) for 30 minutes. Proper controls to be included for each sample are:
    a. An autofluorescence control: 10 µl diluent in place of monoclonal antibody, followed by steps 12 - 16.
    b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin (Sigma Product No. F-6522) at the same concentration as test antibody followed by steps 12 -16.
12. Add 2 ml of diluent to all tubes.
13. Pellet cells by centrifugation at 500 x g for 10 minutes.
14. Remove supernatant by careful aspiration.
15. Repeat steps 12 - 14.
16. Resuspend cells in 0.5 ml of 2% paraformaldehyde. Analyze in a flow cytometer according to manufacturer's instructions.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific binding of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein which has no reactivity with human cells. It should be isotype-matched to the antibody and of the same concentration and F/P molar ratio as the antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.

References

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