Technical Data Sheet

Biotin Mouse Anti-Human PCNA

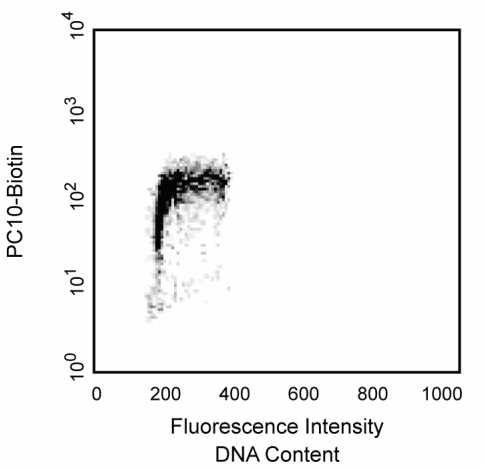
Product Information

Material Number:	**555567**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	PC10
Isotype:	Mouse IgG2a, κ
Reactivity:	QC Testing: Human
Storage Buffer:	Aqueous buffered solution containing ≤0.09% sodium azide.

Description

The **P**roliferating **C**ell **N**uclear **A**ntigen (PCNA) was initially identified as a nuclear antigen in proliferating cells and was subsequently described as a subunit for DNA polymerase δ. PCNA protein levels peak during the S-phase of the cell cycle, at which time it forms a complex with the p21 inhibitor. PCNA is almost undetectable in other phases of the cycle. Because of its unique expression, PCNA has been extensively used in studies associating the prognosis of tumor progression and neoplastic proliferation. Human PCNA has been reported to be 262 amino acids with an apparent molecular weight of 36 kDa.

This antibody is routinely tested by flow cytometric analysis. Other applications were tested at BD Biosciences Pharmingen during antibody development only or reported in the literature.



Profile of peripheral blood lymphocytes analyzed on a FACScan (BDIS, San Jose, CA)

Preparation and Storage

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.
The antibody was conjugated with biotin under optimum conditions, and unreacted biotin was removed.
Store undiluted at 4° C and protected from prolonged exposure to light. Do not freeze.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested

Recommended Assay Procedure:

1. Harvest, count and pellet cells following standard procedures.
   Note: PCNA is expressed by proliferating cells. Using resting cells (eg, unstimulated PBMC) may give negative results.
2. While vortexing, add 5 ml cold 70% - 80% ethanol dropwise into the cell pellet (1-5 x 10e7 cells). Incubate at -20°C for at least 2 hours. These fixed cells can be stored at -20°C for up to 60 days prior to staining.
3. Wash twice with 30-40 ml staining buffer (PBS with 1% FBS, 0.09% NaN3), centrifuge for 10 minutes at 200g.
4. Resuspend the cells to a concentration of 1 X 10e7/ml.

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5. Transfer 100 µl (1 X 10e6 cells) cell suspension into each sample tube.
6. Add 20 µl of properly diluted anti-PCNA antibody according to the protocol into the tubes above. Mix gently.
7. Incubate the tubes at room temperature (RT) for 20-30 minutes in the dark.
8. Wash with 2 ml of staining buffer at 200g for 5 minutes.
9. Aspirate the supernatant.
10. If using directly conjugated anti-PCNA, proceed to step 13.
11. If using purified anti-PCNA, add 50 µl of diluted secondary antibody (eg, cat. no. 555988), if using Biotin conjugated anti-PCNA, add 50µl of SAV-PE (Cat. No. 554061), to each sample tube and incubate at RT for 30 minutes in the dark.
12. Repeat steps 8 & 9.
13. Add 0.5 ml of staining buffer to each tube. If using FITC conjugated anti-PCNA or secondary antibody, add 10 µl of Propidium Iodide Staining Solution (Cat. No. 556463) to each tube; for PE conjugated anti-PCNA or secondary antibody, add 20 µl BD Via-Probe™ Cell Viability Solution (Cat. No. 555816) to each tube.
14. Proceed to flow cytometric analysis.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554061	Streptavidin PE	0.5 mg	(none)
555747	Biotin Mouse IgG1 κ Isotype Control	100 tests	MOPC-21

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.

References

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