Technical Data Sheet

# PE Mouse Anti-Human PCNA Set

## Product Information

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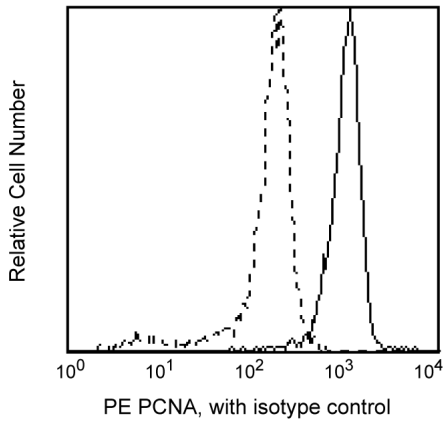
| **Material Number:** | **556031** |
| **Size:** | 100 tests |
| **Reactivity:** | QC Testing: Human |
| **Component:** | **51-32555X** |
| **Description:** | PE Anti-Human PCNA |
| **Size:** | 100 tests (1 ea) |
| **Vol. per Test:** | 20 µl |
| **Clone Name:** | PC10 |
| **Isotype:** | Mouse IgG2a, κ |
| **Storage Buffer:** | Aqueous buffered solution containing BSA and ≤0.09% sodium azide. |
| **Component:** | **51-37085X** |
| **Description:** | PE Mouse IgG2a, κ Isotype Control |
| **Size:** | 100 tests (1 ea) |
| **Clone Name:** | MOPC-173 |
| **Isotype:** | Mouse (BALB/c) IgG2a, κ |
| **Storage Buffer:** | Aqueous buffered solution containing BSA and ≤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.




***Profile of PCNA reactivity on permeabilized MOLT-4 cells 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 R-PE under optimum conditions, and unconjugated antibody and free PE were removed.
Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

## Application Notes

**Application**

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| Intracellular staining (flow cytometry) | Routinely Tested |

**Recommended Assay Procedure:**

1. Harvest, count and pellet cells following standard procedures.

**BD Biosciences**

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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 10^7 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 10^7/ml.
5. Transfer 100 µl (1 X 10^6 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 50 µl 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.

## Product Notices

1. This antibody has been optimized and preassayed with its matched isotype control to be used at the recommended volume of 20 ul/test. Titration of the reagents or substituting with other (non-matched) isotype control is NOT recommended.
2. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
3. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.
4. 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.
5. Source of all serum proteins is from USDA inspected abattoirs located in the United States.

## References


Garcia RL, Coltrera MD, Gown AM. Analysis of proliferative grade using anti-PCNA/cyclin monoclonal antibodies in fixed, embedded tissues. Comparison with flow cytometric analysis. *Am J Pathol.* 1989; 134(4):733-739. (Clone-specific: Flow cytometry)

Landberg G, Tan EM, Roos G. Flow cytometric multiparameter analysis of proliferating cell nuclear antigen/cyclin and Ki-67 antigen: a new view of the cell cycle. *Exp Cell Res.* 1990; 187(1):111-118. (Clone-specific: Flow cytometry)

Mathews MB, Bernstein RM, Franza BR Jr, Garrels JI. Identity of the proliferating cell nuclear antigen and cyclin. *Nature.* 1984; 309(5966):374-376. (Clone-specific: Flow cytometry)

Ogata K, Ogata Y, Nakamura RM, Tan EM. Purification and N-terminal amino acid sequence of proliferating cell nuclear antigen (PCNA)/cyclin and development of ELISA for anti-PCNA antibodies. *J Immunol.* 1985; 135(4):2623-2627. (Clone-specific: Flow cytometry)

Schlatt S, Weinbauer GF. Immunohistochemical localization of proliferating cell nuclear antigen as a tool to study cell proliferation in rodent and primate testes. *Int J Androl.* 1994; 17(4):214-222. (Clone-specific: Flow cytometry)