Technical Data Sheet

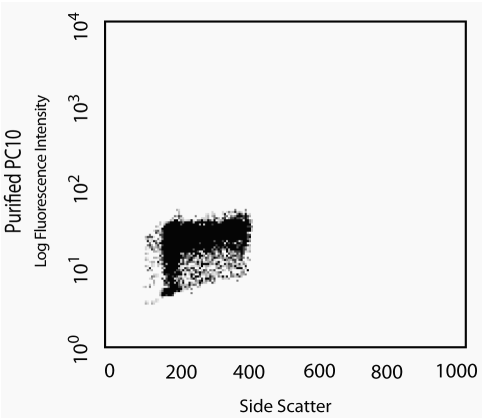
Purified Mouse Anti-Human PCNA

Product Information

Material Number:	**555566**
Size:	0.1 mg
Concentration:	0.5 mg/ml
Clone:	PC10
Immunogen:	Not Reported
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.



Profile of methanol-fixed MOLT-4 cells analyzed on a BD FACScan™ (BDIS, San Jose, CA)

Immunofluorescent staining of HeLa (ATCC CCL-2) cells. *Cells were seeded in a 96 well imaging plate (Cat. No. 353219) at ~10,000 cells per well. After overnight incubation, cells were stained using the alcohol perm protocol and the anti-PCNA antibody. The second step reagent was Alexa Fluor® 555 goat anti-mouse IgG (Invitrogen). The image was taken on a BD Pathway™ 855 Bioimager using a 20x objective. This antibody also stained A549 (ATCC CCL-185) and U-2 OS (ATCC HTB-96) cells. The Triton™ X-100 perm protocol is not recommended for use with this antibody. (See Recommended Assay Procedure).*

Preparation and Storage

Store undiluted at 4°C.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

Application Notes

Application

Intracellular staining (flow cytometry)	Routinely Tested
Immunohistochemistry-formalin (antigen retrieval required)	Tested During Development
Bioimaging	Tested During Development

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Recommended Assay Procedure:

Bioimaging

1. Seed the cells in appropriate culture medium at ~10,000 cells per well in a BD Falcon™ 96-well Imaging Plate (Cat. No. 353219) and culture overnight.
2. Remove the culture medium from the wells, and fix the cells by adding 100 µl of BD Cytofix™ Fixation Buffer (Cat. No. 554655) to each well. Incubate for 10 minutes at room temperature (RT).
3. Remove the fixative from the wells, and permeabilize the cells using either BD Perm Buffer III, 90% methanol, or Triton™ X-100:
   a. Add 100 µl of -20°C 90% methanol or Perm Buffer III (Cat. No. 558050) to each well and incubate for 5 minutes at RT.

   OR

   b. Add 100 µl of 0.1% Triton™ X-100 to each well and incubate for 5 minutes at RT.
4. Remove the permeabilization buffer, and wash the wells twice with 100 µl of 1× PBS.
5. Remove the PBS, and block the cells by adding 100 µl of BD Pharmingen™ Stain Buffer (FBS) (Cat. No. 554656) to each well. Incubate for 30 minutes at RT.
6. Remove the blocking buffer and add 50 µl of the optimally titrated primary antibody (diluted in Stain Buffer) to each well, and incubate for 1 hour at RT.
7. Remove the primary antibody, and wash the wells three times with 100 µl of 1× PBS.
8. Remove the PBS, and add the second step reagent at its optimally titrated concentration in 50 µl to each well, and incubate in the dark for 1 hour at RT.
9. Remove the second step reagent, and wash the wells three times with 100 µl of 1× PBS.
10. Remove the PBS, and counter-stain the nuclei by adding 200 µl per well of 2 µg/ml Hoechst 33342 (e.g., Sigma-Aldrich Cat. No. B2261) in 1× PBS to each well at least 15 minutes before imaging.
11. View and analyze the cells on an appropriate imaging instrument.

***Immunohistochemistry*:** The fixation time of the tissue is critical to maintain the integrity of the antigen. Fixation periods longer than 8 hrs will reduce the chance for detecting PCNA in the tissue.

Suggested Companion Products

Catalog Number	Name	Size	Clone
555988	FITC Goat Anti-Mouse IgG/IgM	0.5 mg	Polyclonal
555571	Purified Mouse IgG2a, κ Isotype Control	0.1 mg	G155-178
553999	Biotin Goat Anti-Mouse Ig (Multiple Adsorption)	0.5 mg	Polyclonal
550946	Streptavidin HRP	50 mL	(none)
550524	Retrievagen A (pH 6.0)	1000 mL	(none)
353219	BD Falcon™ 96-well Imaging Plate		(none)
554655	Fixation Buffer	100 mL	(none)
558050	Perm Buffer III	125 mL	(none)
554656	Stain Buffer (FBS)	500 mL	(none)

Product Notices

1. Since applications vary, each investigator should titrate the reagent to obtain optimal results.
2. This antibody has been developed and certified for the bioimaging application. However, a routine bioimaging test is not performed on every lot. Researchers are encouraged to titrate the reagent for optimal performance.
3. Triton is a trademark of the Dow Chemical Company.
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. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.

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, Immunohistochemistry)

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. (Biology)

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. (Biology)

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: Immunohistochemistry)

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