PCNA [PC10]

Concentrated and Prediluted Monoclonal Antibody
901-3255-060223

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3255 A, C	0.1, 1.0 mL	1:100	Da Vinci Green
Predilute	API 3255 AA	6.0 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 3255 G	6.0 mL	Ready-to-use	N/A

Intended Use:
For In Vitro Diagnostic Use
PCNA [PC10] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of PCNA protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
Proliferating cell nuclear antigen (PCNA) is necessary for DNA synthesis and is an accessory protein for DNA polymerase alpha, which is elevated during the G1/S phase of the cell cycle. PCNA forms a ring around a portion of DNA, serving to anchor the various DNA replication and repair proteins and to regulate proliferation throughout the cell cycle. In staining applications, the PCNA antibody exhibits nuclear staining (1,2). PCNA is overexpressed in many cancer types, and overexpression is correlated with cancer virulence with studies showing that PCNA is directly related to the degree of tumor differentiation, stage of cancer and the prognosis of cancer. PCNA-targeting peptides were shown to inhibit the growth or to induce apoptosis in neuroblastoma, prostate cancer, breast cancer, bladder cancer, and multiple myeloma (3,4).

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human; others not tested
Clone: PC10
Isotype: IgG2a/kappa
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: PCNA
Cellular Localization: Nuclear
Positive Tissue Control: Breast cancer and prostate cancer
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative
Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Probe: Incubate for 10 minutes at RT with a secondary probe.
Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.
Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.
Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (Ventana BenchMark ULTRA):
AVI3255 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Template/Detection: OptiView DAB IHC
Pretreatment Protocol: CC1 32 minutes
Peroxidase: Pre Primary Peroxidase
Primary Antibody: 32 minutes, 36°C

Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (5)

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Precautions (Cont'd):
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (6)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:
1. Guo JL, *et al.* Evaluation of Clinical Significance of Endoglin Expression During Breast Cancer and Its Correlation with ER and PCNA. Eur Rev Med Pharmacol Sci. 2017 Dec; 21(23):5402-7.
2. Bologna-Molina R, *et al.* Comparison of the value of PCNA and Ki-67 as markers of cell proliferation in ameloblastic tumor. Med Oral Patol Oral Cir Bucal. 2013 Mar; 18(2):e174–9.
3. Shemesh A, *et al.* NKp44-Derived Peptide Binds Proliferating Cell Nuclear Antigen and Mediates Tumor Cell Death. Front Immunol. 2018; 9:1114.
4. Zhao H, *et al.* Interaction of proliferation cell nuclear antigen (PCNA) with c-Abl in cell proliferation and response to DNA damages in breast cancer. PLoS One. 2012; 7(1):e29416.
5. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
6. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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