Novocastra™ Liquid Mouse Monoclonal Antibody Proliferating Cell Nuclear Antigen

Product Code: NCL-L-PCNA

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Proliferating cell nuclear antigen (PCNA) from all vertebrate species and insects.
Clone	PC10
Ig Class	IgG2a
Antigen Used for Immunizations	Rat PCNA induced in the protein A expression vector pR1T2T (Wassem and Lane, 1990).
Hybridoma Partner	Mouse myeloma (Sp2/0-Ag14).
Preparation	Liquid tissue culture supernatant containing 15 mM sodium azide. Volume as indicated on vial label.
Effective on Frozen Tissue	No
Effective on Paraffin Wax Embedded Tissue	Yes. PCNA immunoreactivity can be detected in material fixed in a wide range of fixatives including formalin (buffered and unbuffered), methacarn and Bouin's reagent. The time of fixation can markedly affect the intensity of PCNA immunoreactivity. High temperature antigen unmasking using 10 mM citrate buffer (pH 6.0) may improve staining on overfixed tissues, but due to increased sensitivity using this technique, care must be taken with the interpretation of results. Staining is reduced (and may be abolished) if sections are baked onto glass slides. Air drying overnight onto 3-aminopropyltriethoxysilane (Apes) coated slides is recommended.
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:100–1:200. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Typical working dilution 1:250–1:500. Effective in indirect flow cytometry.
Positive Controls	Immunohistochemistry: Tonsil or reactive lymph node. Western Blotting: MCF-7 cell line. Flow Cytometry: A549 cell line.
Staining Pattern	Nuclear.
Storage and Stability	Store liquid antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. Prepare working dilutions on the day of use.
General Overview	Proliferating cell nuclear antigen (PCNA) is a 36 kD molecule which is highly conserved between species. PCNA functions as a co-factor for DNA polymerase delta in S phase and also during DNA synthesis associated with DNA damage repair mechanisms. The PCNA molecule has a half-life in excess of 20 hours and therefore, may also be detected in non-cycling cells eg those in G0 phase. NCL-PCNA is of value in the immunohistochemical analysis of cells in a proliferating fraction.
General References	Zdunek M and Korobowicz E. Polish Journal of Pathology. 51 (2): 77–81 (2000). Tsujimura A, Takano Y, Oka T, et al.. Nippon Hinyokika Gakkai Zasshi. 88 (6): 618–623 (1997). Coltrera M D and Gown A M. Journal of Histochemistry and Cytochemistry. 39 (1): 23–30 (1991). Hall P A, Levison D A, Woods A L, et al.. Journal of Pathology. 162: 285–294 (1990). Wassem N H and Lane D P. Journal of Cell Science. 96: 121–129 (1990). Takasaki Y, Deng J-S and Tan E M. Journal of Experimental Medicine. 154: 1899–1909 (1981).

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Instructions for Use

High Temperature Antigen Unmasking Technique for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).
Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available).
Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).
Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

** In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ MANUFACTURER'S INSTRUCTIONS.