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Human PCNA Ready-To-Use IHC Kit

Cat. No.: IHC0123H

Size: 50T (including a control slide)
Sample Type: FFPE tissue
Storage and Stability: Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

General Information

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8℃
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8℃
4	Blocking Buffer	3 ml	RTU	2-8℃
5	Primary Antibody (Human PCNA Mouse mAb)	6 ml	RTU	2-8℃
6	Secondary Antibody (HRP-Goat anti-Mouse IgG pAb)	6 ml	RTU	2-8℃
7	Chromogen Component A	0.3 ml	RTU	-20℃
8	Chromogen Component B	0.3 ml	RTU	-20℃
9	Counter Staining Reagent	5 ml	RTU	RT
10	Differentiation Reagent	6 ml	RTU	RT
11	Mounting Media	5 ml	RTU	RT
12	Control slide (Human colon)	1 slide	RTU	RT
13	Datasheet	1 copy		

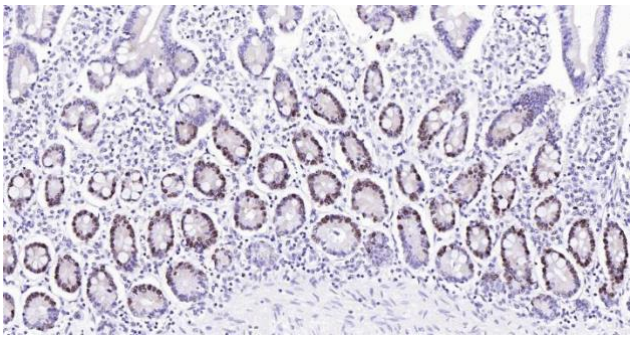
Background

PCNA (polymerase delta auxiliary protein) is essential for DNA replication and is involved in DNA excision and mismatch repair pathways. PCNA binds to the CDK inhibitor p21, the structure-specific endonucleases Fen1 and XPG, and DNA cytosine 5-methyltransferase (MCMT). PCNA is a potentially useful marker of cells with proliferative potential and for identifying the proliferation status of tumor tissue (i.e. relevant to prognosis). PCNA is a marker for cells in early G1 phase and S phase of the cell cycle. PCNA is found in the nucleus and is a cofactor of DNA polymerase delta, and acts as a homotrimer and helps increase the processing of leading strand synthesis during DNA replication. In response to DNA damage, PCNA is ubiquitinated and is involved in the RAD6-dependent DNA repair pathway. Two transcript variants encoding the same protein have been found for PCNA. Pseudogenes of PCNA have been described on chromosome 4 and on the X chromosome.

Synonyms

Cyclin; DNA polymerase delta auxiliary protein; HGCN8729; MGC8367; Mutagen-sensitive 209 protein; Pcna/cyclin; PCNAR; Polymerase delta accessory protein; Proliferating Cell Nuclear Antigen.

Validation Data



Immunohistochemical analysis of paraffin embedded human small intestine tissue slide using IHC0123H (Human PCNA IHC Kit).

Immunohistochemistry Protocol

1. **Deparaffinization And Rehydration**
   Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.
2. **Antigen Retrieval**
   Add 100×**Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.
3. **Block Endogenous Peroxidase**
   Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.
4. **Serum Blocking**
   Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.
5. **Primary Antibody Incubation**
   Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Human PCNA Mouse mAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.
6. **Secondary Antibody Incubation**
   Incubate slides with 2-4 drops of **HRP-Goat anti-Mouse IgG pAb** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.
7. **Signal Development**
   Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.
8. **Counterstain**
   Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.
9. **Dehydration Sheet**
   Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

Notes

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4℃ after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as "IHC0123H, Bioss Antibodies".
Citation example: "Human tissue sections using Human PCNA IHC Kit (IHC0123H, Bioss Antibodies) were stained for PCNA according to the manufacturer's instructions.