VitroView™ *In Situ* PCNA Detection Kit (150 tests)

Cat. No. VB-4003L

Introduction

Proliferating cell nuclear antigen (PCNA) is a DNA sliding clamp that increase the processivity of DNA polymerase delta and is essential for replication. PCNA expression increases from the late G1 phase through the S-phase of the cell cycle. PCNA protein expression is a well-accepted marker of proliferation. VitroView™ In Situ PCNA Detection Kit detects PCNA expression in tissue cells during cell proliferation. This Kit is optimized for immunohistochemical staining of PCNA in formalin-fixed paraffin-embedded (FFPE) sections, frozen sections, and cultured or isolated cells on slides. The antibody against PCNA works in human, mouse, and rat with strong specific staining and lower background.

Application

In situ detection of Proliferating cell nuclear antigen (PCNA) positive proliferation cells in in human, mouse, and rat cells/tissues.

Kit Components

1. 10× Ag retrieval solution 150 mL
2. RTU block buffer 15 mL
3. RTU anti-PCNA antibody 15 mL
4. RTU biotinylated anti-rabbit secondary antibody 15 mL
5. RTU Streptavidin-HRP 15 mL
6. PCNA positive control FFPE slides 5 slides

Note: RTU=ready-to-use

Storage

Store at 4 ºC.

Material Needed But NOT Supplied with the Kit

1. Xylene and ethanol
2. Distilled or deionized water
3. 30% hydrogen peroxide
4. 10 mM phosphate-buffered saline (PBS), pH 7.4
5. Triton X-100
6. Mini PAP pen
7. DAB Substrate Kit (Cat#: VB-6003 or VB-6003E)
8. Hematoxylin (Cat#:VB-6004)
9. Mounting media

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Protocol

1. Preparation of Slides

A. Cell Lines

1) Grow cultured cells on sterile glass cover slips or slides overnight at 37 ºC
2) Wash briefly with PBS
3) Fix as desired. Possible procedures include:
   a. 20 minutes with 10% formalin in PBS (keep wet)
   b. 10 minutes with ice cold methanol, allow to air dry
   c. 10 minutes with ice cold acetone, allow to air dry
4) Wash in PBS

B. Frozen Sections

1) Snap frozen fresh tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryomolds. Store the frozen tissue block at -80 ºC until ready for sectioning.
2) Transfer the frozen tissue block to a cryotome cryostat (e.g. -20 ºC) prior to sectioning and allow the temperature of the frozen tissue block to equilibrate to the temperature of the cryotome cryostat.
3) Section the frozen tissue block into a desired thickness (typically 5-10 µm) using a cryostat.
4) Place the tissue sections onto glass slides suitable for immunohistochemistry (e.g. Superfrost).
5) Sections can be stored in a sealed slide box at -80 ºC for later use.
6) Before staining, warm slides at room temperature for 30 minutes and fix in ice cold acetone or ice cold methanol for 10 minutes. Air dry for 30 minutes.
7) Wash in PBS.

C. Paraffin Sections

1) Deparaffinize sections in xylene, 3×5 minutes.
2) Hydrate with 100% ethanol, 2×2 minutes.
3) Hydrate with 95% ethanol, 2×2 minutes.
4) Rinse in distilled water.
5) Follow procedure for BrdU antigen retrieval as required.

2. Antigen retrieval

Formalin-fixed tissues require an antigen retrieval step before immunohistochemical staining can proceed.

1) Dilute 10×Ag Retrieval Solution to 1×Ag Retrieval Solution by adding 90 mL ddH_2O into 10 mL of 10×Ag Retrieval Solution.

2) Bring slides to a boil in 1×Ag Retrieval Solution at a sub-boiling temperature for 10-15 minutes. Cool slides on bench top for 30 minutes.

Note: Do not use this pretreatment with frozen sections or cultured cells that are not paraffin-embedded.

3. Staining Procedure

1) Rinse sections in PBS-Triton X-100 (0.025%) for 2 minutes×2.

2) **Serum Blocking**: Incubate sections with 2-3 drops of RTU normal goat serum for 30 minutes to block non-specific binding of immunoglobulin.

3) **Primary Antibody**: Incubate sections with primary antibody (Rabbit IgG) at appropriate dilution in antibody dilution buffer (CAT#: VB-6002) for 1-2 hours at room temperature or overnight at 4 °C. Rinse in PBS.

4) **Peroxidase Blocking** (optional): incubate sections in 0.3% hydrogen peroxide in PBS for 10 minutes at room temperature. Rinse in PBS.

5) **Secondary Antibody**: incubate sections with 2-3 drops of RTU biotinylated anti-Rabbit secondary antibody for 30 minutes at room temperature.

6) Rinse in PBS for 2 minutes×3.

7) **Detection**: incubate sections with 2-3 drops of RTU streptavidin-HRP for 30 minutes at room temperature.

8) Rinse in PBS for 2 minutes×3.

9) **Chromogen/Substrate**: incubate sections with 3 drops of DAB solution for 2-8 minutes. Monitor signal development under a microscope.

Note: DAB solution is made by mixing 20 µL of DAB stock solution and 20 µL of stable H_2O_2 solution with 1 mL of DAB enhancer buffer (dark-brown stain) or DAB buffer (brown stain) which are included in DAB Substrate Kit (CAT#: VB-6003 or VB-6003E).

10) Rinse in distilled water 2 minutes×2.

11) **Counterstain**: For using Hematoxylin Nuclear Counterstaining Kit (CAT#: VB-6004), incubate sections with 3 drops of RTU hematoxylin solution for 1-2 minutes. Rinse in tap water 2 minutes×2.

12) Dehydrate through 75% ethanol for 2 min, 95% ethanol for 2 min, and 100% ethanol for 3 minutes×2. Clear in xylene for 5 minutes×2.

13) Coverslip with mounting medium.

4. IHC Troubleshooting

High background staining

Possible Cause	Solution
Endogenous peroxidase activity was incompletely blocked.	Incubate sections in 0.3% hydrogen peroxide in methanol or PBS for 10-30 minutes at room temperature.

Deparaffinization was incomplete.	Prepare new sections and deparaffinize according to standard laboratory protocol using fresh xylene or xylene substitute.
Inadequate rinsing of slides.	Gently rinse slide with wash buffer bottle and place in wash bath for 5 minutes. Gentle agitation of the wash bath may increase effectiveness.
Over-development of substrate.	Reduce incubation time.
Dehydration of specimen during staining.	Keep section wet.

Negative staining on positive slides

Possible Cause	**Solution**
Steps in the staining protocol were performed in incorrect sequence.	Repeat the procedure.
Primary or secondary antibody incubation steps were omitted.	Repeat the procedure.
Labile antigens were destroyed.	Use freshly-cut slides. Use a paraffin wax with a melting temperature at 55-58 ºC. Temperature of wax used for embedding should not exceed 60 ºC.
Specimen was improperly fixed and/or processed.	Check manufacture's specifications regarding recommended fixative
Specimen dehydrated during staining.	Repeat the procedure by following the manufacture's protocol.

Weak staining on all slides

Possible Cause	**Solution**
Specimen retained excess liquid after rinsing steps.	Remove excess liquid after rinsing steps.
Incubation times were insufficient.	Prolong incubation time.
Substrate prepared improperly.	Check compatibility of buffer ingredients with enzyme and substrate-chromogen reagents. Repeat staining.
Deparaffinization was incomplete (staining may be accompanied by high background).	Prepare new sections and deparaffinize according to standard laboratory protocol using fresh xylene or xylene substitute.

Warning: DAB is a possible carcinogen. Please take necessary precautions.

Note: This product is intended for research purposes only. This product is not intended to be used for therapeutic or diagnostic purposes in humans or animals. Avoid contact with eyes, skin and clothing. Do not ingest. Wear gloves.

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