



**VitroView™ *In Situ* Ki67 IHC/DAB Detection Kit**  
**(For 50 Tests)**  
**SKU#: VB-4002D**

**Description**

The Ki-67 antigen is a large nuclear protein (345, 395 kDa). The Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2, and mitosis), but is absent from resting cells (G0). Therefore the expression of the human Ki-67 protein is strictly associated with cell proliferation and is not detectable during DNA repair processes. The expression of Ki67 is extensively used as a proliferation marker. The VitroView™ *In Situ* Ki67 IHC/DAB Detection Kit detects Ki67 expression in tissue cells during cell proliferation. This Kit is optimized for one step immunohistochemical staining of Ki67 in formalin-fixed paraffin-embedded (FFPE) sections, frozen sections, and cultured or isolated cells on slides. Our antibody against Ki67 works in human, mouse, rat, sheep, rabbit, horse, cow, dog, pig, monkey and Chinese hamster tissue

**Application**

*In situ* detection of Ki67 positive proliferation cells in human, mouse, rat, sheep, rabbit, horse, cow, dog, pig, monkey and Chinese hamster tissue.

**Contents**

1. 10×Ag retrieval solution-----50 ml
2. RTU Block buffer-----5 ml
3. RTU anti-Ki67 antibody-----5 ml
4. RTU polymeric peroxidase conjugated secondary antibody-----5 ml
5. DAB stock solution (40×) -----0.5 ml
6. DAB buffer-----20 ml
7. Ki67 positive control FFPE slides-----2 slides

Note: RTU=ready-to-use

**Reagents and Material Required but Not Provided**

- Xylene and ethanol
- Distilled or deionized water
- 30% hydrogen peroxide
- 10 mM phosphate-buffered saline (PBS), pH 7.4
- Triton X-100
- Mini PAP Pen
- Hematoxylin (Cat#:VB-6004)
- Mounting Media

**Storage**

Store at 2-8°C.

**Protocol:**

**1. Preparation of Slides**

**A. Cell Lines**

- Grow cultured cells on sterile glass cover slips or slides overnight at 37 ° C
- Wash briefly with PBS

- 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
- Wash in PBS

### B. Frozen Sections

- 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°C until ready for sectioning.
- Transfer the frozen tissue block to a cryotome cryostat (e.g. -20°C) prior to sectioning and allow the temperature of the frozen tissue block to equilibrate to the temperature of the cryotome cryostat.
- Section the frozen tissue block into a desired thickness (typically 5-10 µm) using the cryotome.
- Place the tissue sections onto glass slides suitable for immunohistochemistry (e.g. Superfrost).
- Sections can be stored in a sealed slide box at -80°C for later use.
- 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.
- Wash in PBS

### C. Paraffin Sections

- Deparaffinize sections in xylene, 3×5min.
- Hydrate with 100% ethanol, 2×2min.
- Hydrate with 95% ethanol, 2×2min.
- Rinse in distilled water.
- Follow procedure for pretreatment as required.

## 2. Antigen retrieval

Prepare 100ml of 1×Ag retrieval working solution by mixing 10 ml of 10× Ag Retrieval Solution with 90 ml of ddH<sub>2</sub>O. Place the slides in a coplin jar containing the 1×Ag retrieval working solution and heat in a microwave oven for 3 min to bring slides to boil. Other sources of heat such as pressure cooker or hot water bath may be used. Maintain at a sub-boiling temperature for 10 minutes. Slowly 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 2×2min
- 2) **Serum Blocking:** incubate sections with 3-4 drops of RTU Block buffer for 30 minutes to block non-specific binding of immunoglobulin.
- 3) **Primary Antibody:** incubate sections with RTU anti-Ki67 antibody for 1-2 hour 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) **Detection:** incubate sections with 3-4 drops of RTU polymeric peroxidase conjugated secondary antibody for 30 minutes at room temperature.
- 6) Rinse in PBS for 3×2min.
- 7) **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 mixture of 25 µl of DAB stock solution with 1ml of DAB buffer.
- 8) Rinse in distilled water 2×2 min
- 9) **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 2×2 min.

- 10) Dehydrate through 75% ethanol for 2 min, 95% ethanol for 2 min, and 100% ethanol for 2x3min. Clear in xylene for 2x5min.
- 11) Coverslip with mounting medium.

## 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 fresh cutting slides.<br>Use a paraffin wax with a melting temperature ~55-58°C. Wax used for embedding should not be exceed 60 °C. |
| 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.  |
| 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.