VitroView™ 1-Step Anti-Rabbit Polymer-Based IHC Kit
(100 tests)

SKU#: VB-6025

Description
Immunohistochemistry (IHC) is a method of detecting the presence of specific proteins in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. IHC is widely used in the diagnosis of abnormal cells and basic research to understand the distribution and localization of biomarkers and differentially expressed proteins in different parts of a biological tissue.
Polymerizing enzymes and attaching these polymers to antibodies is a new technology. This technology has been applied to both primary antibodies and detection systems. The VitroView™ Polymer Based 1-step IHC Kit utilizes a novel polymerization technology to prepare polymeric HRP-linker antibody conjugates.
The advantages of this technology include: 1) Biotin-Free; 2) High sensitivity; 3) Low background; 4) Reduction of steps and time; 5) Ready-to-use; 6) Simplified multiple labeling.

Application
Immunohistochemistry for detecting a primary antibody made in Rabbit.

Contents
1. RTU normal horse serum----------10ml
2. RTU polymeric peroxidase anti-rabbit secondary antibody----------10ml
   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
- Primary antibody
- DAB Substrate Kit (Cat#: VB-6003 or VB-6003E)
- 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 º 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

Most formalin-fixed tissues require an antigen retrieval step before immunohistochemical staining can proceed. Heat-mediated and enzymatic antigen retrievals are common methods.

- **For Citrate:** Bring slides to a boil in 10 mM sodium citrate buffer, pH 6.0; maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.
- **For EDTA:** Bring slides to a boil in 1 mM EDTA, pH 8.0: follow with 15 minutes at a sub-boiling temperature. No cooling is necessary.
- **For TE:** Bring slides to a boil in 10 mM TE/1 mM EDTA, pH 9.0: then maintain at a sub-boiling temperature for 18 minutes. Cool at room temperature for 30 minutes.
- **For Pepsin:** Digest for 10 minutes at 37°C.

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 normal horse 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 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 anti-Rabbit 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 enhancer buffer (dark-brown stain) or DAB buffer (brown stain) which are included in DAB Substrate Kit (CAT#: VB-6003 or VB-6003E).
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 tape 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 2×5min.
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. Use a paraffin wax with a melting temperature ~55-58ºC. Wax used for embedding should not be 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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