



**VitroView™ LSAB IHC Kit (Rabbit IgG)
(For 100 Tests)**

SKU: VB-6017

Description

Immunohistochemistry (IHC) is a widely used technique for detecting and localizing specific proteins, biomarkers, and other antigens in cells and tissue sections. IHC is commonly used in biomedical research and pathology to study the distribution, localization, and expression of proteins within biological tissues.

The VitroView™ LSAB IHC Kit (Rabbit IgG) is designed for immunohistochemical detection of primary antibodies raised in rabbit using the labeled streptavidin-biotin (LSAB) detection method. The kit uses a biotinylated anti-rabbit secondary antibody to bind the rabbit primary antibody, followed by a streptavidin-horseradish peroxidase (streptavidin-HRP) conjugate for signal detection.

The LSAB method provides enhanced IHC sensitivity compared with conventional direct peroxidase-conjugated antibody detection. Multiple biotin molecules on the biotinylated secondary antibody enable binding of streptavidin-HRP, amplifying the immunohistochemical signal and improving visualization of target antigens.

The VitroView™ LSAB IHC Kit is supplied with ready-to-use (RTU) reagents, providing a convenient and consistent workflow for routine immunohistochemistry on tissue sections.

Key Features

- Designed for rabbit primary antibodies
- Based on the labeled streptavidin-biotin (LSAB) IHC method
- Provides enhanced signal sensitivity through biotin-streptavidin signal amplification
- Includes ready-to-use (RTU) detection reagents
- Suitable for immunohistochemical staining of tissue sections
- Compatible with HRP-based chromogenic IHC detection
- Convenient workflow for research and routine IHC applications

Application

- Immunohistochemistry (IHC) for detecting primary antibodies raised in rabbits.
- The kit can be used to detect and localize target proteins and biomarkers in appropriately prepared tissue sections.

Contents

Component	Volume
RTU Normal Goat Serum	10 mL
RTU Biotinylated Anti-Rabbit Secondary Antibody	10 mL
RTU Streptavidin-HRP	10 mL

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 (SKU: VB-6003 or VB-6003E)
- Hematoxylin (SKU:VB-6004)
- Mount Media

Storage

Store at 2-8°C.

Protocol:

1. Preparation of Slides

A. Cell Lines

- Grow cultured cells on sterile glass coverslips 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

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 the benchtop 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 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 (SKU: 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 3-4 drops of RTU biotinylated anti-Rabbit secondary antibody for 30 minutes at room temperature.
- 6) Rinse in PBS for 3 × 2 min.
- 7) **Detection:** incubate sections with 3-4 drops of RTU streptavidin-HRP for 30 minutes at room temperature.
- 8) Rinse in PBS for 3×2min.
- 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 prepared by mixing 25 µl of DAB stock solution with 1ml of DAB enhancer buffer (dark-brown stain) or DAB buffer (brown stain), which are included in the DAB Substrate Kit (SKU: VB-6003 or VB-6003E).
- 10) Rinse in distilled water 2×2 min
- 11) **Counterstain:** For using Hematoxylin Nuclear Counterstaining Kit (SKU: VB-6004), incubate sections with 3 drops of RTU hematoxylin solution for 1-2 minutes. Rinse in tap water 2×2 min.
- 12) Dehydrate through 75% ethanol for 2 min, 95% ethanol for 2 min, and 100% ethanol for 2 × 3 min. Clear in xylene for 2×5min.
- 1) Coverslip with mounting medium, such as VitroView™ Permanent Mounting Medium (SKU#VB-8003).

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 the slide with the wash buffer bottle and place it in the 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.
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Negative staining on positive slides

Possible Cause	Solution
The steps in the staining protocol were performed in the 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 paraffin wax with a melting temperature ~55-58°C. Wax used for embedding should not exceed 60 °C.
Specimen was improperly fixed and/or processed.	Check manufacturer's specifications regarding recommended fixative
Specimen dehydrates during staining.	Repeat the procedure by following the manufacturer'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.