



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

**SKU: VB-6017D**

**Description**

Immunohistochemistry (IHC) is a method for detecting the presence of specific proteins in cells of a tissue section by exploiting the principle that antibodies bind 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. LSAB IHC Kit is based on the labeled streptavidin-biotin (LSAB) method. This method utilizes a biotinylated secondary antibody that links primary antibodies to streptavidin-peroxidase conjugate. In this method, a single primary antibody is subsequently associated with multiple biotin molecules. Therefore, an increase in sensitivity is achieved compared to direct peroxidase-conjugate methods.

**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   |
| DAB stock solution (40×)                        | 0.75 ml |
| DAB buffer                                      | 30 ml   |
| RTU hematoxylin solution                        | 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
- Mounting 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-freeze 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  $3 \times 2$  min.
- 7) **Detection:** incubate sections with 3-4 drops of RTU streptavidin-HRP for 30 minutes at room temperature.
- 8) Rinse in PBS for  $3 \times 2$  min.
- 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 25  $\mu$ l of DAB stock solution with 1ml of DAB buffer (dark-brown stain).
- 10) Rinse in distilled water  $2 \times 2$  min.
- 11) **Counterstain:** Incubate sections with 3 drops of RTU hematoxylin solution for 1-2 minutes. Rinse in tap water  $2 \times 2$  min.
- 12) Dehydrate through 75% ethanol for 2 min, 95% ethanol for 2 min, and 100% ethanol for  $2 \times 3$  min. Clear in xylene for  $2 \times 5$  min.
- 1) Coverslip with mounting medium, such as VitroView™ Permanent Mounting Medium (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 slide with wash buffer bottle and place it 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 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 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.