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

**SKU: VB-6015**

**Description:**
The VitroView™ LSAB IHC Kit (Mouse IgG) is a ready-to-use immunohistochemistry (IHC) detection system for detecting mouse primary antibodies in tissue sections. The kit utilizes the labeled streptavidin-biotin (LSAB) method to provide sensitive and reliable chromogenic IHC detection.
Immunohistochemistry is widely used to detect and localize specific proteins and biomarkers in tissue sections. By exploiting the specific binding between antibodies and tissue antigens, IHC enables researchers to evaluate the distribution, localization, and relative expression of target proteins in biological tissues.
The VitroView™ LSAB IHC Kit uses a biotinylated anti-mouse secondary antibody followed by a streptavidin-HRP conjugate. The biotin-streptavidin interaction creates a highly efficient detection system for mouse primary antibodies.
Compared with direct enzyme-conjugated antibody methods, the LSAB approach can provide enhanced detection sensitivity because multiple biotin molecules associated with the secondary antibody can bind to streptavidin-HRP, increasing the amount of enzyme associated with the antibody-antigen complex.
The ready-to-use reagents help simplify IHC workflows and reduce reagent preparation time.

**Key Features**
- Designed for mouse primary antibodies (Mouse IgG)
- LSAB-based IHC detection technology
- Ready-to-use (RTU) reagents
- Biotinylated anti-mouse secondary antibody
- Streptavidin-HRP detection system
- Sensitive chromogenic IHC detection
- Suitable for routine immunohistochemistry on tissue sections
- Convenient 100-test format

**Application**
- The VitroView™ LSAB IHC Kit (Mouse IgG) is intended for immunohistochemical detection of antigens using mouse primary antibodies.
- It can be used in research applications for detecting and localizing biomarkers and specific proteins in tissue sections.

**Kit Contents**

| Component | Volume |
|---|---|
| RTU Normal Horse Serum | 10 mL |
| RTU Biotinylated Anti-Mouse Secondary Antibody | 10 mL |
| RTU Streptavidin-HRP | 10 mL |

**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-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 horse serum for 30 minutes to block non-specific binding of immunoglobulin.
3) **Primary Antibody**: incubate sections with primary antibody 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-mouse secondary antibody for 30 minutes at room temperature.
6) Rinse in PBS for 3x2min.
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.
13) 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 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. |

**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. |

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| | Use paraffin wax with a melting temperature ~55-58ºC. Wax used for embedding should not exceed 60 ºC. |
| 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.