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

SKU: VB-6015D

Description

Immunohistochemistry (IHC) is a widely used technique for detecting and localizing specific proteins, biomarkers, and other antigens in cells and tissue sections. By utilizing the specific binding of antibodies to target antigens, IHC enables researchers to evaluate the distribution, localization, and expression of proteins within biological tissues. IHC is widely used in biomedical research, pathology, and the study of disease-related biomarkers.

The VitroView™ LSAB IHC/DAB Kit (Mouse IgG) is designed for the immunohistochemical detection of primary antibodies raised in mice. The kit is based on the labeled streptavidin-biotin (LSAB) detection method and provides a sensitive, convenient workflow for HRP-based chromogenic IHC staining.

The LSAB detection system uses a biotinylated anti-mouse secondary antibody to bind the mouse primary antibody, followed by streptavidin-HRP to generate an amplified detection signal. Multiple biotin molecules associated with the secondary antibody provide binding sites for streptavidin-HRP, resulting in increased signal intensity compared with conventional direct peroxidase-conjugated antibody detection methods.

For convenient chromogenic detection, the kit includes a DAB (3,3-diaminobenzidine) substrate system. In the presence of HRP, DAB produces an insoluble brown reaction product at the site of the target antigen, allowing visualization of protein expression under a standard light microscope. A ready-to-use hematoxylin counterstain is also included to provide clear visualization of tissue morphology and cellular structures.

The VitroView™ LSAB IHC/DAB Kit provides the essential reagents for mouse primary antibody-based IHC with DAB chromogenic detection, making it suitable for routine immunohistochemistry and research applications.

Key Features

- Designed for mouse primary antibodies (Mouse IgG)
- Based on the labeled streptavidin-biotin (LSAB) detection system
- Provides enhanced IHC sensitivity through biotin-streptavidin signal amplification
- Includes streptavidin-HRP for enzymatic signal detection
- Includes DAB substrate for brown chromogenic visualization
- Includes ready-to-use hematoxylin for tissue counterstaining
- Convenient ready-to-use (RTU) detection reagents
- Suitable for HRP/DAB immunohistochemistry
- Designed for 100 IHC tests

Application

- Immunohistochemistry (IHC) for detecting primary antibodies raised in mice.
- The kit is suitable for detecting and localizing target proteins and biomarkers in appropriately prepared tissue sections using HRP/DAB chromogenic detection.

Contents

Component	Volume
RTU Normal Goat Serum	10 mL
RTU Biotinylated Anti-Mouse 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 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 dry air.
- 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 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 3×2min.
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 made by mixing 25 µl of DAB stock solution with 1ml of DAB buffer.
10) Rinse in distilled water 2×2 min.
11) **Counterstain**: Incubate sections with 3 drops of RTU hematoxylin solution for 1-2 minutes. Rinse in tape 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 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 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