VitroVivo Biotech
Make Tissues, Cells, Genes and Molecules Visible

VitroView™ Anti-Mouse Immunofluorescenc Staining Kit
(Green, 50-100 slides)

SKU#: VB-6100

Description:
The VitroView™ Anti-Mouse Immunofluorescence Staining Kit is an essential tool for immunochemical techniques, specifically designed to facilitate the precise detection and localization of a wide range of antigens within different tissue types and cell preparations. This user-friendly kit is optimized for use with primary antibodies raised in mice, ensuring accurate and reliable results.

Key Advantages:

1. Enhanced Sensitivity: Achieve high sensitivity in antigen detection, enhancing the quality of your results.
2. Minimal Background Interference: Reduce background noise to a minimum, ensuring clear and reliable data.
3. Streamlined Workflow: Save time and effort with a simplified procedure that minimizes the number of steps required.
4. Easy Multiple Labeling: Facilitate multiple labeling experiments, making your research more efficient.

Specifications

Unit Size	1 kit
Target Species	Mouse
Conjugates and Color of Fluorescence	FL488 Conjugated Goat anti Mouse IgG (Far Red)
Excitation/Emission for FL488 dye	496/519 nm
Host Species	Goat
Application	Immunofluorescence staining for detecting a primary antibody made in mouse.

Kit Contents:

- RTU Normal Goat Serum ----------10ml
- FL488 Conjugated Goat anti Mouse IgG (1mg/ml)----------50µl
- Aqueous Anti-fade Mounting Medium with Dapi----------1.5 ml×2

Storage
Store at 2-8 °C.

Reagents and Material Required but Not Provided

- Xylene and ethanol for FFPE samples
- Distilled or deionized water
- 10 mM phosphate-buffered saline (PBS), pH 7.4
- Triton X-100
- Mini PAP Pen

- Primary antibody
- Antibody Dilution Buffer (SKU#: VB-6002)
- BSA
- Antigen retrieval reagents

Protocol

1. Preparation of Slides

A. Cell Lines

- Grow cultured cells on chamber slides, sterile glass cover slips or slides overnight at 37°C.
- Briefly wash with PBS.
- Choose one of the following fixation methods (To achieve optimal results, it may be necessary to optimize the fixation methods).
  a. Fix in 10% formalin in PBS for 20 minutes (keep wet).
  b. Immerse in ice-cold methanol for 10 minutes and allow to air dry.
  c. Immerse in ice-cold acetone for 10 minutes and allow to air dry.
- Wash slides in PBS.

B. Frozen Sections

- Snap-freeze fresh tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, then embed them in OCT compound in cryomolds. Store the frozen tissue block at -80°C until ready for sectioning.
- Before sectioning, equilibrate the frozen tissue block to the temperature of the cryotome cryostat (e.g., -20°C).
- Section the frozen tissue block into the desired thickness (typically 5-10 µm) using the cryotome.
- Place the tissue sections onto glass slides suitable for immunohistochemistry (e.g., Superfrost).
- Store sections in a sealed slide box at -80°C for later use.
- Before staining, allow slides to warm to room temperature for 30 minutes and then 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, 3×5 minutes.
- Hydrate with 100% ethanol, 2×2 minutes.
- Hydrate with 95% ethanol, 2×2 minutes.
- Rinse in distilled water.
- Follow the required pretreatment procedure.

2. Antigen Retrieval

Most formalin-fixed tissues require antigen retrieval before proceeding with immunohistochemical staining. Common methods include heat-mediated and enzymatic antigen retrievals. Choose the appropriate method:

a. For Citrate: Bring slides to a boil in 10 mM sodium citrate buffer, pH 6.0, and maintain at a sub-boiling temperature for 10 minutes. Cool slides on the benchtop for 30 minutes.
b. For EDTA: Bring slides to a boil in 1 mM EDTA, pH 8.0, and follow with 15 minutes at a sub-boiling temperature. No cooling is necessary.
c. For TE: Bring slides to a boil in 10 mM TE/1 mM EDTA, pH 9.0, and then maintain at a sub-boiling temperature for 18 minutes. Cool at room temperature for 30 minutes.
d. 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

a. Rinse sections in PBS-Triton X-100 (0.025%) for two 2-minute cycles.
b. Serum Blocking: Incubate sections with 2-4 drops of ready-to-use (RTU) normal goat serum for 30 minutes to block non-specific immunoglobulin binding.
c. Primary Antibody: Incubate sections with primary mouse antibody at the appropriate dilution in antibody dilution buffer (SKU# #: VB-6002) for 1-2 hours at room temperature or overnight at 2-8°C. Rinse in PBS.
d. Detection: Incubate sections with FL488-conjugated Goat anti-Mouse IgG secondary antibody at suitable dilutions (ranging from 1/100 to 1/500) in 0.1% BSA in PBS for 1 hour at room temperature while keeping the samples in the dark.
e. Rinse in PBS for three 2-minute cycles.
f. Counterstaining and mount cover slip with 20-30µl of aqueous anti-fade mounting medium with DAPI. Seal cover slip with nail polish to prevent drying and movement.
g. Visualize and capture the signal using a fluorescence microscope equipped with excitation wavelength filters of 488 nm.
h. Store slides in the dark at 2-8°C.

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.

Precautions: Handle with care. Avoid contact with eyes, skin and clothing. Do not ingest. Wear gloves.