VitroVivo Biotech
Make Tissues, Cells, Genes and Molecules Visible

VitroView™ Immunofluorescence Triple Staining Kit, FL488 Anti-Mouse (Green), FL594 Anti-Rabbit (Red)& FL647 Anti-Goat (far Red) (For 50-100 slides)

SKU#: VB-6301

Description:
Triple immunofluorescence (IF) procedure is needed in order to be able to examine the co-distribution of three different antigens in the same sample. Here we provide reliable convenient VitroView™ Immunofluorescence Triple Staining Kit which can be carried out with 3 primary antibodies raised in the species of mouse, rabbit and goat.

The advantages of this technology include: 1) High sensitivity; 2) Low background; 3) Reduction of steps and time; 4) Simplified multiple labeling.

Specifications

Unit Size	1 kit
Target Species	Mouse, Rabbit and Goat
Conjugates and Color of Fluorescence	FL488 Conjugated Goat anti Mouse IgG (Green) FL594 Conjugated Goat anti Rabbit IgG (Red) FL647 Conjugated Donkey anti Goat IgG (Far Red)
Excitation/Emission for FL488 dye	496/519 nm
Excitation/Emission for FL594 dye	590/617 nm
Excitation/Emission for FL647 dye	647/664 nm
Host Species	Goat and Donkey
Application	Triple IF staining for simultaneous detection of primary antibodies raised in mouse, rabbit and Goat

Kit Contents:
RTU Normal Donkey Serum ----------10ml
FL488 Conjugated Goat anti Mouse (1mg/ml)----------50µl
FL594 Conjugated Goat anti Rabbit (1mg/ml)----------50µl
FL647 Conjugated Donkey anti Goat (1mg/ml) ----------50µl
Aqueous Anti-fade Mounting Medium with Dapi---------- 1.5 ml×2

Storage
Store at 2-8 ℃.

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

Protocol

1. Preparation of Slides

A. Cell Lines

- Grow cultured cells on sterile glass cover slips or slides overnight at 37℃.
- 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℃ until ready for sectioning.
- Before sectioning, equilibrate the frozen tissue block to the temperature of the cryotome cryostat (e.g., -20℃).
- 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℃ 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

1) Rinse sections in PBS-Triton X-100 (0.025%) for two 2-minute cycles.
2) Serum Blocking: Incubate sections with 2-4 drops of ready-to-use (RTU) normal Donkey serum for 30 minutes to block non-specific immunoglobulin binding.
3) Primary Antibody: Incubate sections with a mixture of three primary antibodies (mouse, rabbit and Goat) at the 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) Detection: Incubate sections with a mixture (70-150 µl) of three secondary antibodies raised in different species (with three fluorochromes including FL488 conjugated anti-mouse / FL594 conjugated anti-rabbit /FL647 conjugated anti Goat secondary antibodies) at appropriate dilutions (1/100-1/500) in 0.1% BSA in PBS for 1 hour at room temperature in the dark.
5) Rinse in PBS for three 2-minute cycles.
6) 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.
7) Visualize and capture the signal using a fluorescence microscope equipped with excitation wavelength filters of 488 nm, 594 nm and 647nm.
8) Store slides in the dark at 4°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.