VitroView™ Immunofluorescence Double Staining Kit, FL488 Anti-Mouse (Green) & FL 647 Anti-Rat (Far Red) (For 50-100 slides)

SKU#: VB-6204

Description:

A double immunofluorescence (IF) procedure is needed in order to be able to examine the co-distribution of two different antigens in the same sample.
The advantages of this kit: 1) High sensitivity; 2) Low background; 3) Reduction of steps and time; 4) Simplified double labeling.

Specifications

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

Kit Contents:

RTU Normal Goat Serum ----------10ml
FL488 Conjugated Goat anti Mouse IgG (1mg/ml)----------50µl
FL647 Conjugated Goat anti Rat 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

Protocol

1. Preparation of Slides

A. Cell Lines

- Grow cultured cells on 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

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 goat serum for 30 minutes to block non-specific immunoglobulin binding.
3) Primary Antibody: Incubate sections with a mixture of two primary antibodies (mouse and rat IgG) at the appropriate dilution in antibody dilution buffer (SKU# #: VB-6002) for 1-2 hours at room temperature or overnight at 2-8°C.
4) Rinse in PBS.
5) Detection: Incubate sections with a mixture (70-150 µl) of two secondary antibodies raised in different species (with two fluorochromes including anti-mouse FL488 and anti-rat FL647 secondary antibodies) at appropriate dilutions (1/100-1/500) in 0.1% BSA in PBS for 1 hour at room temperature in the dark.
6) Rinse in PBS for three 2-minute cycles.
7) 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.
8) Visualize and capture the signal using a fluorescence microscope equipped with excitation wavelength filters of 488 nm and 647 nm.
9) 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.