VitroView™ Biotin Donkey Anti-Goat IgG (H+L) Antibody

SKU#: VB-1203

Description

VitroView™ Biotin Donkey Anti-Goat IgG (H+L) Antibody is prepared by labeling high quality donkey anti-goat IgG (H+L) with Biotin. Biotin label is used to subsequent detection by streptavidin for signal amplification.

Specification

Physical State	1.0 mg/ml	Host/Isotype	Donkey
Purification	Immunoaffinity chromatography	Class & Form	Polyclonal/ Whole Antibody
Buffer	PBS, pH 7.4	Type	Secondary Antibody
Preservative	0.02% sodium azide	Tested Species Reactivity	Goat
Stabilizer	0.1% BSA	Target Class	IgG (H+L)
Label	Biotin	Excitation/Emission	-----

Package Size

200 µg in 200 µl buffer

Reconstitution and Storage:

Product is stable for about 12 months at 2-8ºC as an undiluted liquid.

General Protocols

Immunostaining Protocol for Microscopy

1. **Preparation of Slides**

For Cultured Cells

- 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); 2) 10 minutes with ice cold methanol, allow to air dry; 3) 10 minutes with ice cold acetone, allow to air dry
- Wash in PBS

For Frozen Sections

- Snap frozen 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

For Paraffin Sections

- Deparaffinize sections in xylene, 3×5min.
- Hydrate with 00% 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 bench top 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: Generally it is not necessary to pretreat frozen sections or cultured cells with above antigen retrieval protocol.

3. **Staining Procedure**

- Rinse sections in PBS-Triton X-100 (0.025%) for 2×2min
- **Serum Blocking**: incubate sections with 3-4 drops of RTU normal donkey serum for 30 minutes to block non-specific binding of immunoglobulin.
- **Primary Antibody**: incubate sections with primary antibody (goat or goat IgG) at appropriate dilution in antibody dilution buffer (SKU#: VB-6002) for 1-2 hour at room temperature or overnight at 4 °C. Rinse in PBS.

- **Secondary Antibody**: incubate sections with 3-4 drops of RTU biotinylated anti-goat secondary antibody for 30 minutes at room temperature.
- Rinse in PBS for 3x2min.
- **Detection**: incubate sections with 3-4 drops of RTU streptavidin-HRP for DAB visualization or RTU streptavidin-fluorescent dye for fluorescence visualization for 30 minutes at room temperature.
- Rinse in PBS for 3×2min.
- **For DAB visualization**
    1. **Chromogen/Substrate**: incubate sections with 3 drops of DAB solution for 2-8 minutes. Monitor signal development under a microscope
    2. Rinse in distilled water 2×2 min
    3. **Counterstain**: For using Hematoxylin Nuclear Counterstaining Kit (CAT#: VB-6004), incubate sections with 3 drops of RTU hematoxylin solution for 1-2 minutes. Rinse in tape water 2×2 min.
    4. Dehydrate through 75% ethanol for 2 min, 95% ethanol for 2 min, and 100% ethanol for 2x3min. Clear in xylene for 2×5min.
    5. Coverslip with mounting medium.

- **For fluorescence visualization**
    1. Count stain with Dapi (blue fluorescence) or other fluorescence nuclei stain dye (red color) for fluorescent.
    2. Mount with aqueous mounting medium.
    3. The slides can be visualized under fluorescence microscope using the correct filter.
    4. Store slides in the dark at 4°C.