VitroView™ Human on Human (HOH) IHC Kit
(For 100 Tests)

SKU: VB-6041D

Description:
VitroView™ Human on Human (HOH) IHC Kit is specifically designed for the use of human (or humanized) primary antibodies on frozen or paraffin-embedded human tissue sections. This kit overcomes the common challenge of high background staining caused by endogenous human immunoglobulin.

Key Advantages:

- Low background with significant reduction of endogenous human IgG staining
- High sensitivity with excellent signal-to-noise ratio
- Fewer steps and reduced protocol time
- Ready-to-use reagents for ease of application

Application:
Immunohistochemistry for detecting a primary human antibody on human tissue sample.

Content:

1. RTU HOH protein blocking solution----------10ml×2
2. RTU human IgG blocking solution----------10ml
3. RTU biotinylated anti-human secondary antibody----------10ml
4. RTU streptavidin-HRP----------10ml
5. DAB stock solution (40×)---------- 0.75 ml
6. Stable DAB buffer----------30ml
7. RTU hematoxylin solution----------10ml
   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:
Product is stable for about 12 months at 2-8 ºC.

Protocol:
1. Preparation of Slides
A. Cell Lines

- 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).
  b. 10 minutes with ice cold methanol, allow air to dry.

c. 10 minutes with ice cold acetone, allowdry air.

- Wash in PBS.

B. 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

C. Paraffin Sections

- Deparaffinize sections in xylene, 2×5min.
- 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 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: 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 2-4 drops of RTU HOH protein blocking solution for 30 minutes at room temperature to block non-specific binding of immunoglobulin.
3) **Human IgG Blocking**: Mouse IgG Blocking: Incubate tissue sections with 2–4 drops of RTU human IgG blocking solution for 60 minutes at room temperature or overnight at 4°C to effectively block endogenous mouse immunoglobulins. After incubation, rinse sections with PBS.
4) **Primary Antibody**: Incubate sections with primary antibody (human IgG) at an appropriate dilution in RTU HOH protein blocking solution for 30-60 min at room temperature.
   Note: With HOH IHC, the primary antibody incubation is often shorter than usual (e.g., 30 minutes to 1 hour). Overnight incubation can sometimes increase the background.
5) **Peroxidase Blocking**: incubate sections in 0.3% hydrogen peroxide in PBS for 10 minutes at room temperature. Rinse in PBS.
6) **Secondary Antibody**: incubate sections with 2-4 drops of RTU biotinylated anti-human secondary antibody for 30 minutes at room temperature. Rinse in PBS for 3×2min.
7) **Detection**: incubate sections with 2-4 drops of RTU Streptavidin-HRP for 30 minutes at room temperature. Rinse in PBS for 3×2min.

8) **Chromogen/Substrate**: incubate sections with 2-4 drops of DAB solution for 2-8 minutes. Monitor signal development under a microscope. Rinse in distilled water 2×2 min.
   Note: DAB solution is made by mixture of 25 µl of DAB stock solution with 1ml of DAB buffer.
9) **Counterstain**: Incubate sections with 3 drops of RTU hematoxylin solution for 1-2 minutes. Rinse in tape water 2×2 min.
10) Dehydrate by 75% ethanol for 2 min, 95% ethanol for 2 min, and 100% ethanol for 2x3min. Clear in xylene for 2×5min.
11) Mount a coverslip onto a glass slide with Permount or some other suitable organic mounting medium.

Disclaimer:

This user manual serves as a general guideline. Users should adapt procedures based on specific experimental requirements and equipment specifications.