Cytokeratin Rapid IHC Kit  
*For Rapid Immunohistostaining of Cytokeratins*

Cat. No. 28-8783  
*Good for 20 Slides/Tests*  
For Use with Frozen or FFPE Tissues

**INTENDED USE**
For Research Use Only, Not for Use in Diagnostic Procedures.

Cytokeratin RIHC Kit is a sensitive and rapid immunohistochemistry (RIHC) kit that is specific for the detection of cytokeratins in frozen or formalin-fixed, paraffin-embedded (FFPE) tissue sections.

**INTRODUCTION**
The Cytokeratin Rapid IHC Kit was developed for the detection of cytokeratins expressed in histological sections. This standardized and complete kit employs a cocktail of anti-pan-cytokeratin monoclonal antibodies directly conjugated to horseradish peroxidase (HRP) using Invitrogen’s proprietary HRP Polymer Technology. The pan-cytokeratin antibodies react with seven different human cytokeratin isoforms that are specifically expressed in various tissue sections. Cytokeratins are specific to epithelial cells; therefore, it can be used for detection of cells with epithelial origins. Cytokeratin RIHC Kit has a total test-turnaround time of less than 8 minutes, and is simple and easy to use, with all reagents provided in convenient dropper bottles.

**REAGENTS AND MATERIALS PROVIDED**
(Good for 20 Tests/Slides)
A. 1 dropper bottle (3 mL) of Ready-To-Use HRP polymer-conjugated anti-pan-cytokeratin antibodies
B1. 1 dropper bottle (1 mL) of 20X substrate buffer
B2. 1 dropper bottle (1 mL) of 20X DAB chromogen
B3. 1 dropper bottle (1 mL) of 20X hydrogen peroxide
1 package insert with the test and staining protocol

**NOTE:** DAB substrate solution should be made fresh during each assay by adding one drop each of B1, B2, and B3 into 1 mL of distilled water.

**REAGENTS & MATERIALS REQUIRED BUT NOT PROVIDED**
- Acetone
- Mayer’s hematoxylin
- Tris-buffered saline (TBS; 50 mM Tris, pH 7.8, 150 mM NaCl) or PBS
- Distilled or deionized water
- Mounting media, such as Clearmount™ (Invitrogen Cat. No. 00-8010) or Histomount™ (Invitrogen Cat. No. 00-8030)
- Cover slips
- Xylene
- Glass jars
- Humidified chamber at 37 °C
- Pipettor
- Timer
- Light microscope

**STORAGE**
Store at 2-8 °C. Do not freeze the kit.
STABILITY
The kit is stable for at least one year at 4°C. Do not use after expiration date. Reagents should not be used if deterioration or substantial loss of activity is evident.

PRINCIPAL OF TEST
Cytokeratin antigens in frozen sections are specifically recognized and bound by anti-cytokeratin antibodies conjugated with HRP polymer. The DAB substrate solution is added, and the peroxidase (HRP) catalyzes the substrate (Hydrogen Peroxide) to form a brown deposit, which visualizes the location of the antigen.

STAINING PROCEDURE FOR FROZEN TISSUE SECTIONS

| Step 1. Frozen Tissue mount | Mount freshly dissected frozen tissue section (4-5µm in thickness) on slide |
| Step 2. Frozen Tissue fixation | Fix frozen tissue section immediately in acetone for 20 seconds Rinse twice with TBS or PBS |
| Step 3. Antibody incubation | Apply 3-4 drops (100–150 µL) of anti-pan-cyokeratin/HRP conjugates Incubate for 3 minutes at 37 °C Rinse twice with TBS or PBS |
| Step 4. Substrate addition | Add 3-4 drops of DAB substrate solution Incubate for 3 minutes at 37 °C Rinse twice with water |
| Step 5. Counterstaining | Stain with Mayer’s hematoxylin for 10 seconds Rinse twice with water Evaluate the slide prior to permanent mounting |
| Step 6. Coverslip mount | Apply coverslip with mounting media |

STAINING PROCEDURE FOR FORMALIN-FIXED, PARAFFIN-EMBEDDED TISSUE SECTIONS
For Formalin-fixed, Paraffin-embedded Tissue Sections, Pretreatment using HIER is Required

Heat Induced Epitope Retrieval (HIER) Procedure
I. Deparaffinize slides. Tissue sections should be mounted on silane, poly-L-Lysine, or HistoGrip (Cat. No. 00-8050) coated slides.
II. Wash slides with distilled water 3 times for 2 min each.
III. Place a 1L glass (Pyrex) beaker containing 500 mL of 0.01 M citrate buffer (Invitrogen Cat. No. 00-5000) on a hot plate. Heat the buffer solution until it boils. (This step may be prepared before slide deparaffinization, as the buffer may take several minutes to boil).
IV. Put the slides in a slide rack and place in the beaker with boiling buffer. Keep it boiling for 15 minutes.
V. After heating, remove beaker from the hot plate and allow it to cool down for at least 15-20 minutes at room temperature.
VI. Rinse slides with PBS (Invitrogen Cat. No. 00-3000) and begin the staining procedure starting from Step 3. Antibody incubation listed above.

EXPECTED RESULTS
The cytokeratin antibodies used in the kit react with human cytokeratin isoforms that are specific to epithelial cells26-28. Although the Cytokeratin Rapid IHC kit is common for the detection of cytokeratins in frozen sections, the kit performed equivalently well on the formalin-fixed, paraffin-embedded tissue sections. The staining intensity usually reflects the effects of tissue preparation as well as antigen concentration. An intense staining indicates a relatively higher expression of cytokeratins, while a lighter staining is indicative of a lower level of cytokeratin expression.
NOTE: All reagents should be equilibrated to room temperature prior to immunostaining. While doing the anti-cytokeratin incubation, prepare the DAB substrate solution as previously instructed. See note on page 1 under REAGENT AND MATERIALS PROVIDED section.
REFERENCE

TRADEMARKS
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