



Epitomics IHC HRP DETECTION KIT USERMANUAL

Protocol # UM-1200 v3.0
Published March 21, 2006

EPITOMICS TANDEM™ IHC STAINING AND DETECTION KIT USERMANUAL

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2.0 PURPOSE

This kit is intended for *in vitro research purposes* and has been optimized for paraffin embedded formalin fixed tissues. Each kit provides enough reagents to perform up to 50 staining reactions.

3.0 BACKGROUND

Epitomics RabMAb IHC Detection Kit is a complete ready-to-use system, designed to be utilized with Epitomics primary Rabbit Monoclonal antibodies (RabMAbs) for detection of specific target antigens in formalin fixed, paraffin-embedded tissue samples.

When combined with an appropriate RabMab the **IHC Detection Kit** combines the superior antigen recognition of rabbit monoclonals antibodies with novel polymer based HRP or AP labeled secondary antibodies which provide a strong, clean signal.

Every Epitomics Rabbit Monoclonal antibody listed as working in IHC has been tested and QC'd using the same reagents and protocol as provided in this kit.

4.0 MATERIALS AND REAGENTS PROVIDED

Table 1

Reagent	Form provided	Amount provided
Antigen Retrieval solution (1X)	ready-to-use	500 ml
Peroxidase Block solution	ready-to-use	12 ml
Blocking solution	ready-to-use	12 ml
Primary antibody diluent	ready-to-use	15 ml
Negative control rabbit IgG (1X)	ready-to-use	5 ml
Secondary anti-Rabbit HRP	ready-to-use	10 ml
DAB A	ready-to-use	350 ul
DAB B	ready-to-use	10 ml

Note: Storage conditions - please store all other reagents at 4°C.

4.1 NOTES ON MATERIALS

- DAB
 - i. Add 32ul of DAB A to 1ml of DAB B and mix.
 - ii. The DAB working solution is stable for 5 days in 4°C.

5.0 MATERIALS AND REAGENTS REQUIRED

- Xylene
- Ethanol, anhydrous denatured, histological grade (100%, 95%, 70%, 50%)
- Washing buffer:
 - i. TBST washing buffer: (50 mM Tris, pH 7.6, 150 mM NaCl, 0.1% Tween-20) – TBST is Tris Base Saline buffer with 0.1% Tween-20
- Distilled water (dH₂O)
- Mayer's Hematoxylin QS
- Permanent Mounting medium
- Rabbit monoclonal primary antibody

5.1 EQUIPMENT REQUIRED

- Slide Rack
- Aluminum Slide holder
- Rice cooker
- Pipettors and pipet tips of various sizes
- Glass coverslips

6.0 PROCEDURE (Please read through entire procedure before beginning)

1. *Deparaffinization/Rehydration*

- a. Heat slides in an oven at 65 °C for 1 hour.
- b. De-paraffinize/hydrate using the following series of 5 minute washes:
 - i. Two Xylene washes
 - ii. Two 100% ethanol wash
 - iii. One 95% ethanol wash
 - iv. One 70% ethanol wash
 - v. One 50% ethanol wash
 - vi. One Distilled water wash on a shaker

2. *Antigen Retrieval*

- a. Immerse slides into staining dish containing Antigen Retrieval Solution.
- b. Place covered staining dish into the rice cooker. Add 120 mL dH₂O and press “cook”.
- c. When “cook” is turned off (about 20–30 min), unplug the cooker and remove the staining dish to the bench top.
- d. Allow to cool down, without cover, for 20 min.

3. *Staining*

- a. Wash slides with TBST for 5 min on a shaker.
- b. Inactivate endogenous peroxidase by covering tissue with the peroxide block solution for 10 min.
- c. Wash slides three times with TBST (3 min each on a shaker).
- d. Block slides with the blocking solution for 1 hour.
- e. Dilute rabbit monoclonal primary antibody in primary antibody diluent to the recommended dilution (final volume of 100-200ul)
- f. Apply primary antibody to tissue section and incubate overnight in the humidified chamber (4 °C). **NOTE:** 100-200ul can be use to cover one slide
- g. Wash slides three times with TBST (3 min each on a shaker).
- h. Apply secondary HRP-conjugated anti-rabbit antibody (ready-to-use, do not dilute) to each section (final volume of 100-200ul) and incubate for 30 minutes at room temperature.
- i. Wash slides three times with TBST (3 min each on a shaker).

- j. Add freshly prepared DAB substrate to the sections.
- k. Incubate tissue sections with the substrate at room temperature until suitable staining develops (generally 1 - 2 min).
- l. Rinse sections with water.
- m. Counterstain with Mayer's Hematoxylin.
- n. Rinse sections well with water.
- o. Dehydrate samples using two rinses with 100% Ethanol (20 dips per rinse) followed by two rinses with Xylene (30 dips per rinse).
- p. Mount coverslips on slides using Permount medium.

7.0 TROUBLESHOOTING

Table 3 – TroubleShooting

Problem	Possible Cause
No staining	-Steps of the protocol or reagents not used in proper order -Antigens are destroyed -Tissue is incorrectly fixed/ or processed
Weak Staining	-Incomplete deparaffinization (weak staining with high background) -Inadequate epitope retrieval -Inadequate reagents incubation time -Inappropriate tissue fixation method used -Incorrect substrates preparation
High Background	-Incomplete deparaffinization -Incomplete blocking of peroxidase activity -Slides not thoroughly washed -Inappropriate tissue fixation method used -Sections dried during staining procedure -Excessive incubation with substrates

8.0 Single Color Detection Kits

Catalog number	Description
4002-1	Epitomics HRP IHC Detection kit
4003-1	Epitomics AP IHC Detection kit

9.0 Related Products

Description	Catalog #
Various	Tandem™ IHC Staining Kits (including primary RabMAb)
4000-1	Tandem Detection Kit I without RabMAb
4001-1	Tandem Detection Kit II without RabMAb
3051-1	Goat anti-Rabbit IgG polymerized HRP secondary
3052-1	Goat anti-Rabbit IgG polymerized AP secondary
4005-1	Fast Red tablets & Solution

For additional information on Epitomics IHC kits, please refer to: www.epitomics.com