

**RABBIT RAPID STAINING KIT****STOCK NO. QUIK-2**

## Directions for Use

**Background and Principle:**

The introduction of immunohistochemical techniques has ushered a new era of staining into the laboratory based upon sensitive, specific methods.<sup>1,2</sup> Using antigen-antibody relationships, tissue components previously undetected can be precisely identified. Rapid immunohistochemical procedures are desirable in several situations. For example, various research applications involving screening of large amounts of tissues and performance of double or multiple staining procedures. In the Sigma procedure, an appropriately characterized, user supplied, high-affinity primary antibody to the antigen of interest is applied to deparaffinized or frozen hydrated tissue sections. Following a brief incubation and a wash, the section is briefly incubated with a biotinylated secondary antibody against immunoglobulins of the relevant species and washed. The section is then incubated with the ExtrAvidin<sup>®</sup> peroxidase reagent. A stable ExtrAvidin-biotin complex is formed with the bound biotinylated antibody. The sites of antibody deposition are visualized by the addition of freshly prepared substrate, which contains hydrogen peroxide and the chromogen 3-amino-9-ethyl-carbazole (AEC). The bound peroxidase catalyzes the oxidation of the AEC to form a reddish-brown, insoluble precipitate at the antigen sites. Staining is completed, if desired, by counterstaining with hematoxylin, and coverslipping. Compared to conventional PAP and Avidin-biotin peroxidase immunostaining techniques, Sigma's rapid procedure substantially decreases staining time without loss of specificity and sensitivity.

**Reagents Provided:**

**Biotinylated Secondary Antibody, Vial 1:** Goat Anti-Rabbit Immunoglobulins in buffered saline, 0.1% sodium azide added as a preservative.

**Peroxidase Reagent, Vial 2:** ExtrAvidin<sup>®</sup>-Peroxidase conjugate in buffered saline, preservative added.

**Acetate Buffer, Vial 3a:** Acetate buffer, 2.5M, pH 5.0.

**AEC Chromogen, Vial 3b:** 3-amino-9-ethylcarbazole (AEC) in N,N-dimethylformamide.

**Hydrogen Peroxide, Vial 4:** 3% H<sub>2</sub>O<sub>2</sub> in deionized water.

**Precautions:**

Biotinylated Secondary Antibody contains sodium azide. Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

AEC chromogen contains 3-amino-9-ethylcarbazole. AEC is harmful if swallowed, inhaled or absorbed through skin. AEC is a possible carcinogen. Avoid all contact. Wear protective clothing. Wash thoroughly after handling.

### **Reagents and Equipment Required, but Not Provided:**

Phosphate buffered saline, pH 7.4 (available as tablets, Sigma Product No. P-4417)

Deionized water.

Mayers Hematoxylin Solution (Sigma Product No. MHS-1).

Glycerol Gelatin (Sigma Product No. GG-1).

Slides, coverslips (Sigma Product Nos. S-8400 and C-9802).

Humidity chamber (Sigma Product No. H-6644).

Light microscope.

### **Specimen Preparation:**

Tissues fixed in any fixative compatible with the studied antigen and antibody can be used. Cut tissue sections at 4-6 microns. Fixed, frozen sections may also be used. For detection of antigens requiring unmasking, digest formalin fixed material with 0.1% trypsin (Sigma Product No. T-8128) or 0.1% protease (Sigma Product No. P-5147) prior to step 3 in the procedure. Blocking of non-specific protein binding is usually not needed.

**Note:** Since tissue sections have a tendency to fall off during immunohistochemical procedures, Poly-L-Lysine (Sigma Product No. P-8920) may be used as a tissue adhesive.

**Note:** Poly-L-Lysine precoated slides are available as Sigma Product No. P-0425.

### **Controls:**

For the correct interpretation of the staining results it is necessary to run a positive control tissue section known to contain the antigen in question and a negative control test section incubated with a negative control reagent such as buffered saline, appropriately diluted non-immune serum immunoglobulin or irrelevant isolated antibody or absorbed primary antibody.

**Note:** All rinses are with phosphate buffered saline (PBS), pH 7.4. Following incubations slides should be washed gently with PBS from a wash bottle, avoid direct jet of water which may wash off or loosen sections. Carefully wipe each slide free of excess fluid before the application of the next reagent. Avoid touching the tissue section. Be certain to apply enough drops of the reagents to cover the sections. DO NOT allow the tissue sections to dry out at any time during the procedure. It is recommended the incubations be performed in a humidity chamber. All incubations are at room temperature unless otherwise specified. Wash steps can include placing slides in a PBS bath for one minute. Interruption of the staining procedure is possible by leaving the slides in PBS washing baths.

### **Procedure:**

1. Deparaffinize and hydrate paraffin sections to water.
2. Optional: Quench endogenous peroxidase activity with 2 drops 3% hydrogen peroxide (Vial 5) for 5 minutes. Wash and wipe slides.
3. Incubate with rabbit primary antibody, appropriately diluted in PBS with 1% bovine serum albumin for 5 minutes. Wipe off excess reagent. Wash and wipe slides.
4. Apply 2 drops Biotinylated Secondary Antibody (Vial 1) and incubate 5 minutes. Wash and wipe slides.
5. Apply 2 drops Peroxidase Reagent (Vial 2) and incubate 5 minutes. Wash and wipe slides.
6. Prepare Substrate Reagent in a test tube.  
In order add:
  - 4 ml deionized water
  - 2 drops Acetate Buffer (Vial 3a)
  - 2 drops AEC Chromogen (Vial 3b)
  - 1 drop 3% hydrogen peroxide (Vial 4)
7. Apply 2 drops Substrate Reagent incubate up to 3 minutes. Check slide microscopically for adequate chromogen development.

8. When sufficient staining has been achieved, rinse slides in deionized water for 2 minutes. Wipe off excess.
9. Optional: Counterstain with Mayer's Hematoxylin for 1 minute.
10. Optional: Rinse in gently running tap water to "blue" the hematoxylin.
11. Apply glycerol-gelatin or other aqueous mounting media and carefully cover with coverslip.
12. Observe by light microscopy.

**Notes:**

1. Optimal concentration of primary antibody for a given application should be determined by titration.
2. The procedure may be further shortened by running steps 3, 4, 5 and 7 at 37°C.
3. Double application of the substrate reagent enhances staining intensity, but may increase background staining.
4. For frozen sections start at step 3. Quenching of endogenous peroxidase activity may be done by incubation with 0.1% H<sub>2</sub>O<sub>2</sub>, for 5 minutes.
5. Endogenous avidin-binding activity in liver, kidney, brain may be significantly suppressed by sequential 10 minute incubations with 0.1% avidin and 0.01% biotin both in 0.05M Tris buffer, pH 7.6, prior to step 1.<sup>3</sup>
6. It is possible to use other chromogens, e.g. 3,3' diaminobenzidine tetrachloride (DAB, available as tablets, Sigma Product No. D5905 or D-4293).

**Expected Observations:**

Nuclei will be blue, while positively stained elements will be rose-red to brownish-red. Diffuse pale staining, if any, in connective tissue should be ignored as non-specific staining of endogenous peroxidase in eosinophils and erythrocytes.

**References:**

1. Taylor, C.R., "Immunoperoxidase Techniques: Practical and Theoretical Aspects," Arch. Pathol. Lab. Med., **102**, 113 (1978).
2. Guesdon, J.L., et al., "The Use of Avidin-Biotin Interaction in Immunoenzymatic Techniques," J. Histochem. Cytochem., **27**, 1131 (1979).
3. Wood, G.S., and Warnke, R., "Suppression of Endogenous Avidin-Binding Activity in Tissues and its Relevance to Biotin-Avidin Detection Systems," J. Histochem. Cytochem. **29**, 1196, (1981).

**Notes:**