Formalin-Fixed, Paraffin-Embedded Tissue Sections

1. Deparaffinize in xylenes using three changes for 5 minutes each. Hydrate sections gradually through graded alcohols: wash in 100% ethanol twice for 10 minutes each, then 95% ethanol twice for 10 minutes each. Wash in deionized H_2O for 1 minute with stirring. Aspirate excess liquid from slides.

Optional: Antigen unmasking may be performed at this point. Certain antigenic determinants are masked by formalin fixation and paraffin embedding and may be exposed by one of several methods:

a) Heat treatment (recommended method): Place slides in a container and cover with 10 mM sodium citrate buffer, pH 6.0; or with 50 mM glycine-HCl buffer (glycine: sc-29096), pH 3.5, with 0.01% (w/v) EDTA (EDTA: sc-29092). Heat at 95º C for 5 minutes. Top off with fresh buffer and heat at 95º C for 5 minutes (optimal incubation time may vary for each tissue type). Allow slides to cool in the buffer for approximately 20 minutes. Wash in deionized H_2O three times for 2 minutes each. Aspirate excess liquid from slides.

b) Pepsin: Incubate sections for 10–20 minutes in 0.1% pepsin in 0.01 N HCl at room temperature. Wash slides several times in deionized H_2O. Aspirate excess liquid from slides.

c) Saponin: Incubate sections for 30 minutes in 0.05% saponin in deionized H_2O at room temperature. Wash at least three times in PBS. Aspirate excess liquid from slides.

Optional: Incubate for 5–10 minutes in 0.1–1% hydrogen peroxide in deionized H_2O to quench endogenous peroxidase activity. Wash in PBS twice for 5 minutes each.

Immunoperoxidase Staining

1) For immunoperoxidase staining of tissue sections, we recommend the use of either the Santa Cruz Biotechnology, Inc. ABC Staining Systems or the ImmunoCruz™ Staining Systems. The ABC Staining Systems utilize preformed avidin-biotinylated horseradish peroxidase complex as a detection reagent, whereas the ImmunoCruz™ Staining Systems utilize a streptavidin-horseradish peroxidase complex. The ImmunoCruz™ Staining Systems include all secondary reagents in a pre-diluted, ready to use format. Complete research protocols are included with all Staining Systems; brief protocols are given below.

2) All steps are carried out at room temperature in a humidified chamber. Allow all Staining System reagents to reach room temperature prior to use. Tissue sections should not be allowed to dry out at any time during the procedure. Use suction to remove reagents after each step, but avoid drying of specimens between steps. Use sufficient reagents to cover the specimens (approximately 100 µl per slide is usually adequate).

ABC Staining Systems

1. Incubate specimens for 1 hour in 1.5% normal blocking serum in PBS (Buffers and General Solutions). Blocking serum (Normal Sera for Immunohistochemistry) ideally should be derived from the same species in which the secondary antibody is raised. Remove blocking serum from slides.

2. Incubate with primary antibody for 30 minutes at room temperature or overnight at 4º C. Optimal antibody concentration should be determined by titration; recommended range is 0.5–5.0 µg/ml diluted in PBS with 1.5% normal blocking serum. Wash with three changes of PBS for 5 minutes each.

3. Incubate for 30 minutes with biotin-conjugated secondary antibody as provided, or at approximately 1 µg/ml diluted in PBS with 1.5% normal blocking serum. Wash with three changes of PBS for 5 minutes each.
4. Incubate for 30 minutes with avidin biotin enzyme reagent. Wash with three changes of PBS for 5 minutes each.
5. Incubate in peroxidase substrate as provided for 30 seconds–10 minutes, or until desired stain intensity develops. Individual slides should be monitored to determine the proper development time. Wash sections in deionized H_2O for 5 minutes. If desired, counter-stain in Gill´s formulation #2 hematoxylin (sc-24973) for 5–10 seconds. Immediately wash with several changes of deionized H_2O.
6. Dehydrate through alcohols and xylenes as follows: Soak in 95% ethanol twice for 10 seconds each, then 100% ethanol twice for 10 seconds each, then xylenes three times for 10 seconds each. Wipe off excess xylene. Immediately add 1–2 drops of permanent mounting medium (e.g., Clarion sc-24942), cover with a glass coverslip (sc-24975) and observe by light microscopy.

ImmunoCruz™ Staining Systems

1. Incubate specimens for 20 minutes in 1–3 drops of serum block. Aspirate serum from slides.
2. Dilute primary antibody in serum block to 0.5–5.0 µg/ml as determined by titration. Incubate for 2 hours. Rinse with PBS (Buffers and General Solutions) then wash in PBS twice for 2 minutes each on a stir plate. Aspirate excess liquid from slides.
3. Incubate for 30 minutes in 1–3 drops of biotinylated secondary antibody. Wash as above.
4. Incubate for 30 minutes in 1–3 drops of HRP-streptavidin complex. Wash as above.
5. Add 1–3 drops HRP substrate mixture. Develop for 30 seconds–10 minutes, or until desired stain intensity develops. Rinse with deionized H_2O and transfer to a deionized H_2O wash for 2 minutes on a stir plate.
6. Counterstain, dehydrate and mount slides as described under ABC Staining Systems.