Oncogene Science™

IMMUNOPEROXIDASE STAINING PROCEDURE

INTRODUCTION:

OSI® Monoclonal Antibodies and Affinity-Purified Polyclonal Antibodies can be used for localization of antigens in tissue sections by immunoperoxidase staining. A method employing the Streptavidin-Biotin system is recommended. For convenience, appropriate OSI® Immuno-Reporter™ Reagents for immunocytochemistry are available from Oncogene Science. In the immunoperoxidase staining procedure, target tissue sections, either frozen or formalin-fixed and paraffin-embedded, are first incubated with a monoclonal antibody or affinity-purified polyclonal antibody to the antigen of interest. Specifically bound antibody is then visualized by incubation with a biotinylated second-step antibody against immunoglobulins of the relevant species (e.g. Biotinylated Goat Anti-Mouse IgG), followed by incubation with a Streptavidin-Horseradish Peroxidase Conjugate and substrate. Using diaminobenzidine as substrate, positive staining is observed as a brown precipitate.

MATERIALS:

1. Equipment

- **Light microscope and microtome.**

2. Solutions and Reagents

- **Tissue sections:** cryostat-cut frozen sections or formalin-fixed, paraffin-embedded sections (4 to 8 microns thick).
- **OSI® Monoclonal Antibody or Affinity Purified Polyclonal Antibody.**
- **Biotinylated Goat Anti-Mouse, Anti-Rat or Anti-Rabbit IgG.**
- **Streptavidin-Horseradish Peroxidase.**
- **Phosphate buffered saline (PBS):** In 1.0 liter distilled water, dissolve: 8.0 g sodium chloride; 1.3 g dibasic sodium phosphate; 0.2 g monobasic sodium phosphate. Adjust to pH 7.4.
- **PBS-BSA:** PBS with 2% (w/v) bovine serum albumin.
- **10% Normal Goat Serum** in PBS.
- **Diaminobenzidine tetrahydrochloride (DAB):** In 100 ml of PBS dissolve 5 mg DAB (e.g. Sigma Chemicals) and add 0.1 ml of 0.3% hydrogen peroxide. Prepare fresh DAB solution daily.
- **Solvents:** ethanol, acetone and xylene.
- **Mounting Medium** (e.g. Permount, Fisher Scientific).
- **Subbing solution:** 0.3% (w/v) gelatin, 0.05% chromium potassium sulfate in distilled H_2O.

PROCEDURE:

1. Preparation of Sections

A. **Frozen Tissue Sections**

- Clean glass slides with 95% ethanol, treat with subbing solution, and air dry.
- Cut 4 to 8 micron thick cryostat sections of tissue block (frozen in isopentane precooled in liquid nitrogen, embedded in OCT compound in cryomolds, and stored at -70°C).
- Allow frozen sections to come to room temperature (30 minutes).
- Fix slides in cold acetone for 10 minutes, keep refrigerated (or choose other fixation procedure).
- Rinse in three changes of PBS.
- Incubate for 5 to 10 minutes in 0.1% hydrogen peroxide in PBS to quench endogenous peroxidase activity.
- Rinse in PBS.

B. **Formalin-fixed, Paraffin-embedded Tissue Sections**

- Clean glass slides with 95% ethanol, treat with subbing solution, and air dry.
- Cut tissue sections using microtome, and apply to slides.
- Deparaffinize sections in xylene, using three changes, 10 minutes each.
- Hydrate sections gradually through graded alcohols.
- Rinse in distilled H_2O.
- Incubate for 15 minutes in 1.0% hydrogen peroxide to quench endogenous peroxidase activity.

- Rinse in PBS.
- Follow procedure for enzymatic digestion or pretreatment with saponin.

2. **Enzymatic Digestion or Saponin Treatment of Tissue Sections**

Some antigenic determinants are masked by formalin fixation and paraffin embedding. In such cases the antigens often may be exposed by enzymatic digestion or treatment with saponin. Pretreatment with saponin is recommended in cases in which enzymatic digestion is not required. The following protocols are suggested:

- **Pronase:** Incubate sections for 4 to 6 minutes in 0.0025% pronase in Tris buffer, pH 7.6, at room temperature. Terminate digestion by washing in PBS containing 0.2% glycine.
- **Trypsin:** Incubate sections for 5 to 20 minutes in 0.1% trypsin in Tris buffer, pH 7.6, at room temperature. Terminate digestion by incubation with soybean trypsin inhibitor in PBS.
- **Pepsin:** Incubate sections for 30 minutes in 0.1% pepsin in 0.01 N HCl buffer, pH 2.25, at room temperature. Terminate by repeated washing in distilled H_2O.
- **Saponin:** Incubate sections for 30 minutes in 0.05% saponin in distilled H_2O, at room temperature. Wash at least three times in PBS.

3. **Immunoperoxidase Staining**

- Carry out incubations at room temperature or at 4°C in humidified chamber. Use suction to remove reagents after each step, but avoid drying of specimens between steps. Use sufficient reagent to cover the specimen (approximately 20 to 50 µl is usually adequate).
- Incubate specimens with 10% goat serum in PBS, 20 minutes, to suppress non-specific binding of IgG.
- Wash with PBS.
- Incubate with primary antibody (OSI® Monoclonal Antibody or Affinity-Purified Polyclonal Antibody) for 60 minutes at room temperature, or overnight at 4°C.

Overnight incubation is recommended for formalin-fixed, paraffin-embedded sections. Optimal antibody concentration for a given application should be determined by titration; usual range 3 to 30 µg/ml in PBS-BSA.

- Rinse with three changes of PBS.
- Incubate with biotin-conjugated second-step antibody (Biotinylated Goat Anti-Mouse IgG or Biotinylated Goat Anti-Rat IgG for OSI® Monoclonal Antibodies; Biotinylated Goat Anti-Rabbit IgG for OSI® Affinity-Purified Polyclonal Antibodies) for 45 minutes. Optimal antibody concentration for a given application should be determined by titration; usual range 2 to 20 µg/ml in PBS.
- Rinse with three changes of PBS.
- Incubate with Streptavidin-Horseradish Peroxidase for 15 minutes. Streptavidin conjugate concentration for a given application should be determined by titration. Usual range 1:100 to 1:500 in PBS.
- Wash extensively with PBS.
- Rinse in 0.5% Triton X-100/PBS for 30 seconds.
- Incubate in DAB solution for 5 minutes. Check under microscope. If further intensification of staining is required, return to DAB and incubate for additional 1 to 5 minutes.
- Rinse in distilled H_2O.
- Counterstain in hematoxylin if desired.
- Dehydrate through alcohols and xylene.
- Mount coverslip using a permanent mounting medium (e.g. Permount).
- Observe by light microscopy.

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ORDERING INFORMATION

Oncogene Science, Inc. research reagents may be ordered by mail at the address shown at left, by telephone or by fax. Terms are net 30 days F.O.B. Uniondale, NY