# Immunofluorescence-Paraffin Testing Protocol

## Deparaffin/Rehydration

*Do not allow slides to dry at any time during the procedure*

1. Incubate sections in three washes of xylene, 5 minutes each.
2. Incubate sections in two washes of 100% ethanol, 10 minutes each.
3. Incubate sections in two washes of 95% ethanol, 10 minutes each.

Wash sections twice in DI water, 5 minutes each.

## Antigen Unmasking/Epitope Reveal

*Consult the antibody's data sheet for the recommended **or required** unmasking step.*

1. Citrate: Bring slides to a boil in 10 mM sodium citrate buffer, pH 6, then reduce temperature to just below boiling for 10-20 minutes. Cool slides at room temperature for 20 minutes.
2. EDTA: Bring slides to a boil in 1 mM EDTA, pH 7.5-8.5, then reduce temperature to just below boiling for 10-20 minutes. Cool slides at room temperature for 20 minutes.
3. Trypsin: Incubate slides in a 1mg/ml Trypsin-PBS solution for 10 minutes at 37oC.
4. Pepsin: Incubate slides in a 1mg/ml Pepsin-Tris HCl solution, pH 2, for 10 minutes at 37oC or 15 minutes at room temperature.

## Staining

1. Wash sections three times in DI water, 5 minutes each.
2. Incubate sections in 3% hydrogen peroxide for 10 minutes.
3. Wash sections twice in DI water, 5 minutes each.
4. Incubate sections in wash buffer for 5 minutes.
5. Block each section for 1 hour at room temperature.
6. Drain (don't wipe) blocking solution and add recommended amount of primary antibody for recommended amount of time.
7. Drain antibody solution and wash sections three times in wash buffer, 5 minutes each.
8. Link step: dilute biotinylated secondary antibody to manufacturer's specification and add to each section. Incubate at room temperature for 30 minutes.
9. Drain antibody solution and wash sections three times in wash buffer, 5 minutes each.

10. Label step: add avidin-fluorochrome reagent to each section and incubate in the dark, at room temperature, for 30-60 minutes.
11. Drain reagent and wash sections three times in wash buffer, in the dark, 5 minutes each.

## Counterstaining (optional)

1. Counterstain the nuclei/DNA with a ready-to-use PI (red fluorescence) or DAPI (blue fluorescence) reagent. Be sure your counterstain is a different color than your avidin-fluorochrome reagent.
2. Drain reagent and wash sections three times in wash buffer, in the dark, 5 minutes each.

## Mounting

1. Mount coverslip with a drop of anti-fade mounting medium.
2. Examine using a fluorescence microscope with appropriate filters.