NOVUS BIOLOGICALS
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IMMUNOFLUORESCENT STAINING OF PARAFFIN-EMBEDDED TISSUE (IF-P)

CONTENTS

DEPARAFFINIZATION AND REHYDRATION

1. Deparaffinize and rehydrate by immersing the slides through the following wells:

   a. Xylene: three washes 5 minutes each
   b. 100% Ethanol, two washes 10 minutes each
   c. 95% Ethanol, two washes 10 minutes each
   d. 70% Ethanol, two washes 10 minutes each
   e. 50% Ethanol, two washes 10 minutes each
   f. Deionized Water, two washes for 5 minutes

   Tip: Before moving to alcohol grades step, make sure to completely deparaffinize the sections. If the sections still have traces of wax, an additional immersion of 5 minutes in Xylene may be employed.

2. Draw a circle on the slide around the tissue with a hydrophobic barrier pen or with rubber cement.

ANTIGEN RETRIEVAL

1. For antigen retrieval using a microwave, bring the slides to a boil in 10 mM sodium citrate buffer (pH 6.0) and then maintain at a sub-boiling temperature for 10 minutes.

   Note: antigen retrieval conditions may require optimization. Read more about Antigen Retrieval for help determining optimal conditions for your sample.

2. Let the slides cool on the bench-top for 30 minutes.

3. Wash the sections by immersing them in distilled water for 5 minutes.

PERMEABILIZATION AND BLOCKING NON-SPECIFIC BINDING

1. To block endogenous peroxidase activity, quench the tissue sections with 3.0% hydrogen peroxide in methanol for 15 minutes.

   Note: To determine if your sample contains endogenous peroxidase, read more about blocking non-specific binding.

2. Wash the sections in distilled water two times for 5 minutes.

3. To permeabilize the tissue/cells, wash the sections twice for 10 minutes with 1% animal serum in PBS with 0.4% Triton X-100 (PBS-T). The species of the animal serum is dependent on the host of your secondary antibody. (e.g. when using a goat anti-mouse secondary, block with goat serum).

4. Block any non-specific binding by incubating the tissue sections with 5% animal serum in PBS-T for 30 minutes at room temperature.

ANTIBODY STAINING

1. Add primary antibody diluted in 1% animal serum PBS-T and incubate at room temperature for 1-2 hours. Then store overnight at 4°C in humidified chamber. Use the recommended dilution of the antibody specified on the datasheet. If not specified, the typical starting dilution can be 2-5 µg/ml. For more information on primary antibody selection, please read our IHC Primary Antibody Selection Guide.

2. Wash sections twice with 1% serum PBS-T for 10 minutes each.

3. Add secondary antibody diluted in 1% serum PBS-T and incubate at room temperature for 1-2 hours. Use the recommended dilution of the antibody specified on the datasheet.

   Note: For help selecting the optimal secondary antibody, please read our Secondary Antibody Handbook.

4. Wash sections twice with 1% serum PBS-T for 10 minutes each.

DOUBLE OR NUCLEAR LABELING (OPTIONAL)

1. Double Labeling: If using a second primary antibody and appropriately matched secondary, repeat step 5-8.

2. Nuclear Labeling: After application of all primary antibodies, DNA binding dyes such as DAPI can be applied to the slides. After dye incubation, wash the slides once for 5 minutes with PBS.

DETECTION

1. Tap off excess wash and apply one drop of anti-fade mounting medium to the slide.

2. Place a coverslip on the tissue sections and circle the edges of the coverslip with clear fingernail polish to prevent the cells from drying. Allow the polish to air dry.

3. Slides may now be examined under a microscope with the appropriate fluorescent filter sets. Be sure to limit slide exposure to light to prevent photobleaching.

4. Slides can be stored between -20°C and 4°C in a dark slide box or slide book.