NOVUS BIOLOGICALS
a bio-techne brand

CHROMOGENIC IMMUNOHISTOCHEMISTRY STAINING OF PARAFFIN-EMBEDDED TISSUE

CONTENTS

- Deparaffinization and Rehydration
- Antigen Retrieval
- Permeabilization and Blocking Non-Specific Binding
- Antibody Staining
- Detection
- Deparaffinization and Mounting

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. Distilled 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 about 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 at least 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 the primary antibody diluted in 1% animal serum in PBS-T and incubate at room temperature for 1-2 hours. Continue the incubation overnight at 4 °C in a humidified chamber.

   Note: Use the recommended dilution of the antibody specified on the datasheet. If not specified, the recommended starting dilution 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 in PBS-T for 10 minutes each.

3. Add a biotinylated secondary antibody and incubate at room temperature for 1 hour. Use the recommended dilution of the antibody specified on the datasheet. 10.

   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.

DETECTION

1. If using the ABC Method, then add ABC-HRP reagent to each section and incubate at room temperature for 1 hour. Follow manufacturer's guidelines for reagent preparation.

2. Wash sections three times in PBS for 10 minutes each.

   Important: DAB is a carcinogen! Always wear gloves and work in a fume hood when working with DAB. Deactivate and clean work area after use according to manufacturer's instructions. Prepare a working solution of DAB and apply to tissue sections. Monitor the reaction as the chromogenic reaction turns the epitope sites brown (time of color development may vary from few seconds to 10 minutes). Proceed to the next step when the intensity of the signal is appropriate for imaging.

3. Wash the sections twice in distilled water for 2 minutes each.

4. As soon as the sections develop, immerse them in deionized water for 2 minutes each.

5. If nuclear counterstaining is desired, use Hematoxylin according to the manufacturer's instructions. Note: If you are using an aqueous chromogen instead of DAB (i.e. AEC, Fast Red, etc.), skip the following dehydration step and mount in aqueous media instead of organic mounting media.

DEHYDRATION AND MOUNTING

1. Dehydrate tissue sections by moving slides through the following solutions twice for 2 minutes each:

   a. 95% Ethanol, two times for 10 seconds each
   b. 100% Ethanol, two times for 10 seconds each
   c. Xylene, two times for 10 second each

2. Add mounting media to slides and top with coverslips. The DAB reaction is permanent and stable and can be analyzed under a brightfield microscope at any time.