Polymer Staining: FFPE Tissue Protocol

1. Deparaffinize and rehydrate the tissue section. Immerse the slide in each reagent for a minimum of 5 minutes.

    - Xylene
    - Xylene
    - 100% Ethanol
    - 100% Ethanol
    - 95% Ethanol
    - 95% Ethanol

2. Place the slide in Deionized Water (DI). Perform antigen retrieval (see antigen retrieval protocols). If the slide does not require antigen retrieval, then place the slide in DI or wash buffer such as PBST: Phosphate Buffered Saline (1X PBS, pH 7.2 with 0.05% Tween 20) or TBST: Tris Buffered Saline (1X TBS, pH 7.6 with 0.05% Tween 20). Wash the slide twice for three minutes each.

    - Optional: Incubate the slide for 20 minutes in 1X PBS, 0.2% Triton X 100 to enhance reagent penetration (e.g. intracellular proteins).

3. Tap off excess buffer and place the slides on the immunostaining tray. Gently dry around the tissue section prior to each incubation. Do not allow the tissue to dry out during the entire immunostaining procedure.

    - Optional: Use a PAP pen to create a hydrophobic barrier around the tissue section.
      The PAP pen reduces the volume of reagent required to cover the tissue section and keeps the reagent localized over the sample.

    - Optional: Incubate the tissue section with an Fc receptor block for 30 minutes. Wash the slide twice for three minutes each.
      Fc receptors are present on a number of cells such as granulocytes, monocytes, and macrophages. The Fc region of the antibody may bind to the Fc receptor and subsequently result in non-specific binding.

4. Incubate the tissue section with a protein block for 10 minutes. Apply enough reagent to cover the sample. Tap off the protein block; do not wash the slide.
   Use an appropriate protein block to reduce non-specific binding that may result from hydrophobic or ionic interactions.

5. Incubate the tissue section with the primary antibody and controls for 30 minutes to 2 hours at room temperature or overnight at 4°C. Adjust the incubation time as needed. Wash the slide twice for three minutes each.

6. Incubate the tissue section with any necessary reagents to block endogenous molecules, such as endogenous peroxidase or phosphatase, with the appropriate blocking reagent(s) for 10 minutes. Wash the slide twice for three minutes each.
   Each staining system utilizes an enzyme to catalyze the chromogen color development solution. However, the enzyme employed may also be naturally occurring in the tissue specimen. For example, peroxidase is present in red blood cells, muscle, kidney, etc. If utilizing a horse-radish peroxidase enzyme, then block endogenous peroxidase with a hydrogen peroxide solution to prevent non-specific color development.

7. Incubate the tissue section with the polymer reagent(s) for 10-20 minutes according to the manufacturer's data sheet. Wash the slides twice for three minutes each.

8. Select the correct chromogen, such as DAB (3,3' Diaminobenzidine) with the peroxidase enzyme or a designated red chromogen with the alkaline phosphatase enzyme. Incubate the tissue section with the chromogen for 5-10 minutes. Refer to the chromogen data sheet instructions (The sample may require a thorough DI wash prior to the chromogen incubation). Wash the slide with DI for 3-5 minutes.

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9. Counterstain the tissue section with hematoxylin for 2-5 minutes. Wash the slide with DI for 3-5 minutes. Rinse with tap water or an alkaline solution to enhance the counterstain.
   The cell nuclei are generally a purple/blue color after the hematoxylin incubation (low pH) and upon incubation with a basic solution the nuclei turn blue.

10. Dehydrate and clear the tissue section. Refer to the chromogen data sheet. (Some chromogens must air-dry or be dehydrated and cleared faster than usual to prevent chromogen fading)

    - 95% Ethanol, 3 minutes
    - 95% Ethanol, 3 minutes
    - 100% Ethanol, 3 minutes
    - 100% Ethanol, 3 minutes
    - Xylene, 5 minutes
    - Xylene, 5 minutes

11. Coverslip the slide with an appropriate mounting media. Refer to the chromogen data sheet. (The chromogen may require an aqueous or non-aqueous mounting media)