Protocol: Fibrinogen Immunohistochemistry – Mouse, updated 8/30/2024

This protocol is optimized for a variety of tissue section types, including cryosections adhered to slides from OCT-embedded fresh-frozen tissue blocks, free-floating sections, and paraformaldehyde-fixed, paraffin-embedded sections. For paraffin sections, it is crucial to perform heat-induced antigen retrieval prior to immunohistochemical staining, as this step can lead to increased background staining. To mitigate this, careful optimization of the antigen retrieval process is recommended to reduce background levels. In our optimization process, we used spinal cord tissues from experimental autoimmune encephalomyelitis (EAE) mice to confirm the specificity and reliability of fibrinogen antibody staining.

Tissue Preparation and Fixation:

- **Free Floating Sections:** Directly proceed to immunostaining, starting with washing in PBS.
- **Cryosections:**
    - **PFA-Fixed and Sucrose Treated:**
        1. Perform intracardiac perfusion with 4% paraformaldehyde (PFA) in PBS.
        2. Postfix the brain overnight at 4°C in 4% PFA in PBS. (Note: Optimal postfixation time may vary depending on the antigen.)
        3. Immerse the brain in 30% sucrose in PBS until it sinks.
        4. Embed the brain in OCT within a plastic mold and freeze on dry ice.

5. Section the brain at 10 μm using a cryostat and mount on Fisherbrand Superfrost Plus slides.
6. Store the slides in a clean slide box, within a plastic zip bag, at -80°C.

- **Fresh Frozen:**
    1. Perform intracardiac perfusion with PBS.
    2. Embed and freeze the brain as described above.
    3. Embed the brain in OCT within a plastic mold and freeze on dry ice.
    4. Section the brain at 10 μm using a cryostat and mount on Fisherbrand Superfrost Plus slides.
    5. Store the slides in a clean slide box, within a plastic zip bag, at -80°C.

- **Paraffin Sections:**
    - **Deparaffinization and Antigen Retrieval:**
        1. Deparaffinize and rehydrate the tissue sections using a series of xylene and ethanol washes.
        2. Immerse slides in preheated (95-99°C) antigen retrieval solution (e.g., DAKO S1699) for 5 minutes. (Optimal time may vary by tissue type.)
        3. Allow slides to cool in the solution for an additional 10 minutes.
        4. Rinse slides with PBS at room temperature, ensuring they do not dry out.
        5. Proceed to immunostaining.

Immunostaining Procedure:

1. Wash the sections with 0.1% PBST (PBS with 0.1% Triton X-100; Sigma 93443) three times, 5 minutes each.

2. Block for 1 hour with 5% BSA and 0.3% Triton X-100 in PBS at room temperature. (Note: Do not substitute BSA with other blocking agents like serum to avoid increased background.)
3. Dilute primary antibodies in 1% BSA and 0.1% Triton X-100 in PBS:
    - Polyclonal goat anti-fibrinogen, GAM/Fbg/7S (1:200, Nordic-MUbio)
4. Apply primary antibody and incubate overnight at 4°C.
5. Wash three times for 5 minutes each with 0.1% Triton X-100 in PBS.
6. Prepare and apply secondary antibody (diluted 1:500 in 0.1% Triton X-100/PBS) and incubate for 1 hour at room temperature.
7. Wash three times for 5 minutes each in PBS.
8. Apply coverslip using Prolong Gold antifade reagent with DAPI. For long-term storage, keep slides flat at 4°C, protected from light.

Additional Note: For immunohistochemical staining of cryosections, use a humidity chamber containing a water-soaked absorbent cotton or paper towel to maintain moisture.