Protocol: Fibrinogen Immunohistochemistry - Mouse

Citation: Davalos D, Ryu JK, Merlini M, Baeten KM, Le Moan N, Petersen MA, Deerinck TJ, Smirnoff DS, Bedard C, Hakozaki H, Gonias Murray S, Ling JB, Lassmann H, Degen JL, Ellisman MH, Akassoglou K. Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammation. **Nat Commun.** 2012; 3:1227

This protocol is suitable for

- cryosections (10 μm) adhered to slides from blocks embedded in OCT
- free floating sections (30 μm)
- paraformaldehyde-fixed, paraffin-embedded sections (5~10 μm)

  Note: paraffin sections require an antigen retrieval step (heat-induced antigen retrieval) before immunohistochemical staining. Antigen retrieval step can cause the background in the staining. Be sure to test the antigen retrieval step to adjust background level.

1. Tissue and Fixation:

- Free floating sections: proceed with immunostaining step #1 (washing with PBS)

- Cryosections:
  - PFA-fixed – sucrose treated
    a. Intracardiac perfusion with 4% paraformaldehyde (PFA) in PBS
    b. postfix brain overnight at 4 °C in 4% PFA in PBS (optimal postfixation time may vary for each antigen).
    c. Transfer brain to 30% sucrose in PBS until it sinks.
    d. Transfer the brain to plastic mold and freeze the brain blocks in OCT on dry ice.

e. Cut 10 um sections on the cryostat and mount the sections on fisherbrand superfrost plus slides

- After sectioning put the slides into a clean slide box and stored (in a plastic zip bag) at -80 C.
- Fresh Frozen
  a. Intracardiac perfusion with PBS
  b. Transfer the brain to plastic mold and freeze the brain blocks in OCT on dry ice.
  c. Cut 10 um sections on the cryostat and mount the sections on fisherbrand superfrost plus slides
  d. After sectioning put the slides into a clean slide box and stored (in a plastic zip bag) at -80 °C.

1) Allow sections to come to room temperature (~30 min).
2) Mark slide with hydrophobic pen.
3) Immerse slided in 4% PFA (in PBS) for 10 min at 4 °C and wash with PBS 3X 5 min. Proceed with immunostaining step #2 (Blocking with BSA).

- Paraffin Sections:
  - Deparaffinization and antigen retrieval procedure
    a. Deparaffinize and rehydrate tissue sections; place the slides in a rack and perform the following washes using Coplin jar:
       1. xylene 1 – 5 min
       2. xylene 2 – 5 min
       3. xylene 3 – 5 min
       4. 100% Ethanol 10 min
       5. 100% Ethanol 10 min
       6. 95% Ethanol 10 min
       7. 70% Ethanol 10 min
       8. place slides in running tap water to rinse off ethanol. Keep the slides in the tap water until ready to perform antigen retrieval.

b. Fill Coplin jar with preheated (95-99 °C) antigen retrieval solution (DAKO, a modified citrate buffer, S1699)
c. Immerse slides in preheated retrieval solution and incubate for 5 min (optimal incubation time may vary for each tissue type).
d. Allow slides to cool in antigen retrieval solution for an additional 10 min.
e. Rinse slides with PBS at room temperature (It is very important that slides do not dry out).
f. Proceed with immunostaining.

2. Immunostaining:

1) Wash with PBS 3X 5min.
2) Block 1 hour with 5% BSA (AMRESCO, 30% BSA solution) and 0.3% Triton X-100 in PBS at RT.

   Note: *BSA cannot be substituted with other blocking reagents* such as feval bovine serum, normal donkey serum, normal goat serum, etc since serum increases background signal.
3) Prepare primary antibody by diluting as below.
   - Antibody dilution solution: 1% BSA and 0.1% Triton X-100 in PBS.
   - Polyclonal sheep anti-fibrinogen (US Biological, F4200-06, 1:300 dilution)

   Polyclonal rabbit anti-fibrinogen (Dr. Degen, 1:1000 dilution)

   Polyclonal rabbit anti-human fibrinogen (DAKO, A0080, 1:1000 dilution)
4) Apply diluted primary antibody and incubate overnight at 4 °C.
5) Wash with 0.1% Trixon X-100 in PBS 3X 5 min. Prepare secondary antibody as below.
   - Antibody dilution solution: 0.1% Triton X-100 in PBS.
   - 2nd antibody (Jackson ImmunoResearch/ Alexa fluor from Molecular Probes, 1:500, diluted in 0.1% Triton X-100/PBS).
6) Apply secondary antibody and incubate for 1 hour at RT.
7) Wash with PBS 3X 5min.
8) Coverslip with Prolong Gold antifade reagent with DAPI. (For long term storage, store slides flat at 4 °C protected from light)

Note: Place the slides in a humidity chamber (containing a piece of absorbent cotton/paper towel that is soaked with water) for immunohistochemical staining of cryosections