Protocol: Fibrinogen Immunohistochemistry - Human

Citation: Petersen MA, Ryu JK, Chang K-J, Etxeberria A, Bardehle S, Mendiola AS, Kamau-Devers W, Fancy SPJ, Thor A, Bushong EA, Baeza-Raja B, Syme C, Wu MD, Rios Coronado PE, Meyer-Franke A, Yahn S, Pous L, Lee JK, Schachtrup C, Lassmann H, Huang EJ, Han MH, Absinta M, Reich DS, Ellisman MH, Rowitch DH, Chan RJ, Akassoglou K. Fibrinogen activates BMP signaling in oligodendrocyte progenitor cells and inhibits remyelination after vascular damage. **Neuron**, 2017, 96:1003-1012

This protocol is suitable for paraformaldehyde-fixed, paraffin-embedded human tissue sections using the Abcam Mouse Monoclonal Anti-Fibrinogen Antibody (ab58207)

1) Deparaffinization / Rehydration

1) Xylene 1 – 10 min
2) Xylene 2 – 10 min
3) 100% ethanol 1 – 5 min
4) 100% ethanol 2 – 5 min
5) 70% ethanol – 5 min
6) 50% ethanol – 5 min
7) Tap water – 5 min, Keep slides in tap water until ready to perform antigen retrieval

2) Antigen Retrieval

1) Place antigen retrieval solution (DAKO S1700, modified citrate buffer) in Coplin jar into an unheated water bath.
2) Turn water bath on to 95 °C and allow water bath and antigen retrieval solution to come up to 95 °C.
3) Once at 95 °C, immerse slides in preheated retrieval solution and incubate for 60 min.
4) Remove Coplin jar from water bath and allow slides to cool in the antigen retrieval solution at room temperature for an additional 30 min.

5) Rinse slides with PBS at room temperature (It is very important that slides do not dry out).
6) Proceed with immunostaining

3) Immunostaining:

1) Mark slide with hydrophobic pen. (Wipe off PBS around tissue sections and apply pen to dry areas – Keep PBS on the tissue itself to keep hydrated while pen mark dries)
2) Block endogenous alkaline phosphatase and peroxidase activity with Bloxall (Vector Labs SP-6000), 10 minutes at room temperature. Rinse with PBS.
3) Block 1 hour with 2.5% serum, 0.3% Triton X-100 in PBS at RT. Use serum from the same host species as your secondary antibody. (Horse serum included in the ImmPRESS™-AP Anti-Mouse IgG (alkaline phosphatase) Polymer Detection Kit).
4) Dilute Abcam **Mouse** Monoclonal Anti-Fibrinogen Antibody (ab58207) 1:500 in 2.5% serum, 0.1% Triton X-100 in PBS
5) Apply diluted primary antibody and incubate overnight at 4 °C in a humidity chamber (containing a piece of absorbent cotton/paper towel that is soaked with water).
6) Wash with 0.1% Triton X-100 in PBS 3 X 5 min.
7) Apply ImmPRESS™-AP Anti-Mouse IgG (alkaline phosphatase) Reagent (made in horse, ready-to-use) from the Vector ImmPRESS kit (MP-5402) and incubate for 1 hour at RT.
8) Wash with PBS 3 X 5min.
9) Develop with Vector Blue Substrate Kit (Vector, SK-5300) per kit instructions (Typically takes 5-15 minutes to develop, helps to watch reaction under microscope and stop by rinsing slide in PBS)
10) If labeling a second antigen, proceed to step 11. Otherwise, mount in VectaMount AQ (aqueous)
11) If labeling a second antigen, re-block for 30-60 minutes with 2.5% serum, 0.3% Triton X-100 in PBS at RT.
12) Dilute **Rabbit** primary antibody in 2.5% serum, 0.1% Triton X-100 in PBS
13) Apply diluted primary antibody and incubate overnight at 4 °C in a humidity chamber.
14) Wash with 0.1% Triton X-100 in PBS 3 X 5 min.

15) Apply ImmPRESS™ VR Anti-Rabbit IgG HRP Reagent (made in horse, ready-to-use) from the Vector ImmPRESS kit (MP-6401-15) and incubate for 30 minutes at RT.
16) Wash with PBS 3 X 5 min.
17) Develop with ImmPACT NovaRED Peroxidase (HRP) Substrate (Vector, SK-4805) per kit instructions (Typically takes 1-5 minutes to develop, helps to watch reaction under microscope and stop by rinsing slide in PBS).
18) Counterstain if desired if colors are compatible.
19) Mount in VectaMount AQ (aqueous)