Protocol: Fibrinogen – iDisco Mouse

Citation: Merlini M, Rafalski VA, Rios Coronado PE, Gill MT, Ellisman M, Muthukumar G, Subramanian KS, Ryu JK, Syme CA, Davalos D, Seeley WW, Mucke L, Nelson RB, Akassoglou K. Fibrinogen induces microglia-mediated spine elimination and cognitive impairment in an Alzheimer's disease model. **Neuron** 2019, 101:1099-1108.

The protocol is largely based on the iDISCO protocol from the Tessier-Lavigne lab available at: http://idisco.info
Akassoglou Lab specific details are listed below.

Antibodies used with the alternative pre-treatment protocol:

Primary antibodies

NAME	VENDOR & catalog #	DILUTION OF STOCK
Rabbit anti-fibrinogen	Gift from Drs. Jay Degen and Eric Mullins, Dept. of Pediatrics, Cincinnati Children's Hospital, Cincinnati, OH, USA	1:1000
Sheep anti-human fibrinogen	US Biological; F4200-06	1:100
Rat anti-CD31/PECAM-1	BD Bioscience 550274	1:20
Rabbit anti-human β amyloid	IBL-America; 18584	1:100

Secondary antibodies

NAME	VENDOR & catalog #	DILUTION OF STOCK
Alexa647-conjugated donkey anti-rabbit	Jackson ImmunoResearch; 711-605-152	1:500 (for fibrinogen) 1:200 (for β amyloid)
Cy3-conjugated donkey anti-rat	Jackson ImmunoResearch; 712-165-153	1:80 (for CD31)

Alexa488-conjugated donkey anti-sheep	Jackson ImmunoResearch; 713-545-147	1:200 (for fibrinogen)

Note: Rabbit anti-fibrinogen (Degen), sheep anti-human fibrinogen (US Biological) and rabbit anti-human β amyloid also work with methanol pre-treatment protocol. Different secondary fluorophore combinations should also work.

Primary and secondary antibody incubation time for a mouse brain hemisphere is 5 days.

IMPORTANT: Perform all primary and secondary antibody incubation steps using 2-mL Eppendorf tubes. All other steps are performed using 5-mL Eppendorf tubes to ensure sufficient washing, pretreatment, and clearing of the samples.

IMPORTANT: Do not use pointy forceps to handle tissue- use blunt forceps

IMPORTANT: DCM and clearing solution should be made on the day of use. Washing buffers can be made in advance. Methanol solutions can be made one day before.

Perfusion and tissue preparation

Perfuse mice with 20 ml 1X PBS followed by perfusion with 20 ml of 4% paraformaldehyde (PFA) in 1X PBS. Extract tissue and post-fix overnight at 4°C in 4% PFA in PBS. If tissue is kept for longer than 1 day prior, store tissue in 1X PBS + 0.02% sodium azide and store in the dark at 4°C. Before beginning iDISCO, acclimate tissue to room temperature for 1 h and then wash 30 min x 3 times in 1X PBS.

Image acquisition

For large volume imaging of cleared hemispheres, the samples were imaged using a Nikon AZ-100 light-sheet microscope equipped with a Vortran 4-line laser launch (50% laser power), an Andor Zyla 5.5 camera, and a 2 × /0.2 AZ Plan Apo objective lens (UCSF Nikon Imaging Center). For high-resolution, small-volume images an Olympus Fluoview 1000 confocal microscope equipped with a 20 × /0.5 NA objective lens was used.