

## Protocol: Fibrinogen Detection with Two-Photon Imaging

**Citation:** Davalos D, Ryu JK, Merlini M, Baeten KM, Le Moan N, Petersen MA, Deerinck TJ, Smirnov 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

The protocol steps outlined below have been applied to and tested for in vivo two-photon imaging of Alexa dye-conjugated fibrinogen in mouse brain and spinal cord using the following Alexa dye-conjugated fibrinogen molecules:

- Alexa488-conjugated human fibrinogen, 5 mg vial: ThermoFisher Scientific, catalog # F13191
- Alexa594-conjugated human fibrinogen, 5 mg vial: ThermoFisher Scientific, catalog # F13193
- Alexa647-conjugated human fibrinogen, 5 mg vial: ThermoFisher Scientific, catalog # F35200

### 1. Preparation of Alexa dye-conjugated fibrinogen working solution

- a. Prepare 0.1 M sodium bicarbonate solution by dissolving 0.8175 g sodium bicarbonate in 97.3 mL cell culture-grade water
- b. Sterile-filter the bicarbonate solution in sterile conditions, e.g., in tissue culture hood. The sterile-filtered bicarbonate solution can be stored at room temperature for 6 months. Prepare fresh bicarbonate solution every 6 months
- c. Add 3.333 mL of the 0.1 M sodium bicarbonate solution prepared above to a 5-mg vial of Alexa dye-conjugated fibrinogen in sterile conditions /in a tissue culture hood. This yields a 1.5-mg/mL Alexa dye-conjugated fibrinogen working solution
- d. Completely dissolve the Alexa dye-conjugated fibrinogen by rotate-mixing at 37 °C using an incubator shaker set at 200 RPM shielded from light (e.g., wrap the vial in aluminium foil). Under these conditions, the Alexa dye-conjugated fibrinogen should be completely dissolved within 30 min

e. Aliquot the completely dissolved Alexa dye-conjugated fibrinogen under sterile conditions in a tissue culture hood and store at -20 °C. A minimum aliquot volume of 100 µL is recommended: 100 µL of Alexa dye-conjugated fibrinogen is required for 20–30 g mouse body weight per injection as outlined under point 2) below

## 2. Retro-orbital injections of Alexa dye-conjugated fibrinogen

**IMPORTANT:** Alexa dye-conjugated fibrinogen needs to be administered per retro-orbital injection as outlined below at **48 h (injection 1)** and **24 h (injection 2) before** the imaging experiment

a. A volume of 100 µL of Alexa dye-conjugated fibrinogen is required for 20–30 g mouse body weight per injection. Thaw the required number of aliquots of Alexa dye-conjugated fibrinogen at 37 °C using this volume guideline. After thawing, invert the solution/aliquot tube 2–3 times

**IMPORTANT:** Keep the thawed aliquots at **30–37 °C** until ready to inject

b. Under light isoflurane anesthesia, i.e., 1.5–2% isoflurane, inject 100 µL of Alexa dye-conjugated fibrinogen retro-orbitally by inserting a 28-gauge 0.5-mL insulin syringe (Becton Dickinson, catalog # 329461) in the caudal part of the eye

**IMPORTANT:** Performing the retro-orbital injection while the mouse is (lightly) anesthetized mice is critical because potential twitching/jerking of the mouse head may cause retro-orbital vascular damage and, hence, mechanical damage-induced intracerebral extravasation of fibrinogen

c. Perform the imaging experiment 24 h after the second injection (see note about injection time points above) using the 2P excitation laser wavelength and emission filter appropriate for the Alexa dye-conjugated fibrinogen used