

Protocol: Fibrinogen Detection with Two-Photon Imaging

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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