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Version 2

Preparation of JF dye for retro-orbital injection in mice V.2

 In 1 collection

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Protocol status: Working

We use this protocol and it's working. Changed 200ul total to 100ul. If need to do 200ul (200nmol dye), it is done with 100ul per eye

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Funders Acknowledgements:

HHMI

Abstract

How to prepare JF dyes for retro orbital injection.

Materials

STEP MATERIALS

 Dimethyl sulfoxide (DMSO) Merck MilliporeSigma (Sigma-Aldrich) Catalog #D2650

 Pluronic™ F-127 (20% Solution in DMSO) Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3000MP

 PBS Invitrogen - Thermo Fisher

 29G x 12.7mm 0.3ml syringe Ulticare Catalog #09230

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Dye preparation

1 Take an aliquote with [M] 100 Mass Percent of dye from ⚡ -20 °C storage.

2 Add 🧪 20 µL DMSO and do ~10 up and down with the pipeter



Dimethyl sulfoxide (DMSO) Merck MilliporeSigma (Sigma-Aldrich) Catalog #D2650

3 Add 20ul Pluronic F-127 20% in DMSO ~10 up and down with the pipeter



Pluronic™ F-127 (20% Solution in DMSO) Merck MilliporeSigma (Sigma-Aldrich) Catalog #P3000MP

4 Add 60ul 1x PBS ~10 up and down with the pipeter



PBS Invitrogen - Thermo Fisher

5 Drew up to 100ul without bubbles to a 29G syringe



29G x 12.7mm 0.3ml syringe Ulticare Catalog #09230

6 Follow the Retro-orbital injection protocol



Protocol

NAME

Retro-orbital injection of virus or dye in mice

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[Preview](#)

- 6.1 Prepare either virus dilution (5.0×10^{11} – 1.0×10^{12}) with PBS prepare or a **dye aliquot**. Injection is up to 200ul per mouse.
- 6.2 Take one mouse from the cage into an induction chamber and start isoflurane at 3% with flow rate of ~1L/min
- 6.3 After the mouse has slow breathing (~1/s) move the mouse to a nose cone delivery of isoflurane at 1.5% with around the same flow rate.
- 6.4 Expose the eye ball with two fingers (above and below) and make sure the you are not pressing on the trachea and the mouse's breathing is regular. 
- 6.5 Use a 29G syringe at a 30-degree angle beveled downwards until you hit the bone, retract a bit and slowly inject. See cited paper for more details. 

Citation

Tal Yardeni, Michael Eckhaus, H. Douglas Morris, Marjan Huizing, Shelley Hoogstraten-Miller

(Invalid date). Retro-orbital injections in mice. LabAnimal.

[10.1038/labani0511-155](https://doi.org/10.1038/labani0511-155)

[LINK](#)

29G x 12.7mm 0.3ml syringe **Ulticare Catalog #09230**



- 6.6 Remove the needle slowly and press the eyelid shut for a few seconds.
- 6.7 Validating the injection was successful is possible with JF525 by looking at the pee of the animal after it wakes up.
Leaving the animal in a clean cup with kim-wips will collect the pee and it would seem pink.

