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🌐 Preparation of JF dye for retro-orbital injection in mice

📁 In 1 collection

Boaz Mohar¹

¹HHMI Janelia Research Campus

Boaz Mohar

HHMI Janelia Research Campus

ABSTRACT

How to prepare [JF dyes](#) for retro orbital injection.

OPEN ACCESS

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Protocol status: In development
 We are still developing and optimizing this protocol

Created: Jul 25, 2019

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PROTOCOL integer ID:
 26236

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**

Dye preparation

1 Take an aliquote with [M] 100 nmol 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 200ul 1x PBS ~10 up and down with the pipeter

⊗ PBS Invitrogen - Thermo Fisher

5 Drew 200ul 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

CREATED BY

Boaz Mohar

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 (2011). Retro-orbital injections in mice. LabAnimal.

LINK

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

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.