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

📁 In 1 collection

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### ABSTRACT

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

### MATERIALS

#### STEP MATERIALS

- ⊗ Dimethyl sulfoxide (DMSO) **Sigma Aldrich Catalog #D2650**
- ⊗ Pluronic™ F-127 (20% Solution in DMSO) **Sigma Aldrich Catalog #P3000MP**
- ⊗ PBS **Invitrogen - Thermo Fisher**
- ⊗ 29G x 12.7mm 0.3ml syringe **Ulticare Catalog #09230**

### PROTOCOL MATERIALS

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

Step 2

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

Step 3

- ⊗ PBS **Invitrogen - Thermo Fisher** Step 4

- ⊗ 29G x 12.7mm 0.3ml syringe **Ulticare Catalog #09230** Step 5


**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

**Created:** Feb 22, 2024**Last Modified:** Feb 22, 2024**PROTOCOL integer ID:** 95629**Funders Acknowledgement:**

HHMI

## Dye preperation

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) **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) **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**

CREATED BY

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PREVIEW

6.1 Prepare either virus dilution ( $5.0 \times 10^{11}$ - $1.0 \times 10^{12}$ ) 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.