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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

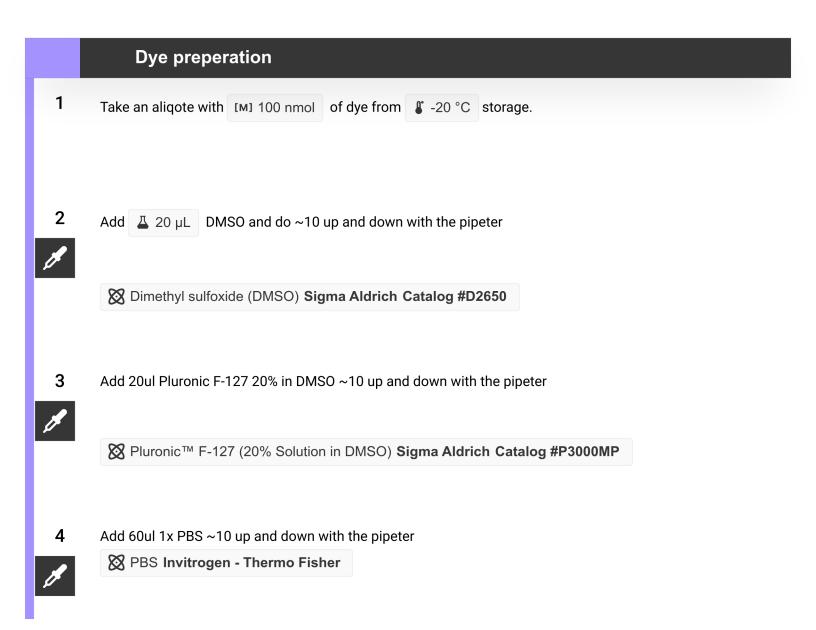
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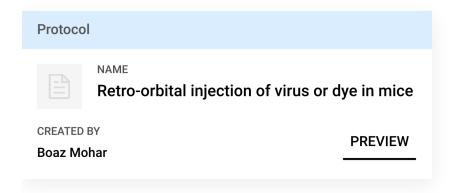
PROTOCOL integer ID: 95629

Funders Acknowledgement:

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- 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



- **6.1** Prepare either virus dilution (5.0e11-1.0e12) with PBS prepare or a dye aliquot. Injection is up to 200ul per mouse.
- 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.

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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/laban0511-155

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.