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

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In Development

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## ABSTRACT


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

## STEPS MATERIALS

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

## Dye preparation

1 Take an aliquote with **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)

by 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)

by Sigma Aldrich

Catalog #: P3000MP


4 

Add 200ul 1x PBS ~10 up and down with the pipeter




**PBS**  
by [Invitrogen - Thermo Fisher](#)


5 Drew 200ul without bubbles to a 29G syringe



**29G x 12.7mm 0.3ml syringe**  
by [Ulticare](#)  
Catalog #: [09230](#)

6 Follow the Retro-orbital injection protocol





Retro-orbital injection of virus or dye in mice  
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[PREVIEW](#)

[RUN](#)

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.



Tal Yardeni, Michael Eckhaus, H. Douglas Morris, Marjan Huizing, Shelley Hoogstraten-Miller (2011). Retro-orbital injections in mice. LabAnimal.  
<http://10.1038/labon0511-155>



29G x 12.7mm 0.3ml syringe  
by 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.



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