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Protocol for mouse perfusion with dye, DAPI staining, and slide preparation

Forked from Protocol for mouse perfusion with dye, DAPI staining, and slide preparation

In 1 collection

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1 Works for me dx.doi.org/10.17504/protocols.io.59jg94n

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MATERIALS

NAME	CATALOG #	VENDOR
Phosphate Buffered Saline	28374	Thermo Fisher Scientific
Paraformaldehyde fixative: 4% paraformaldehyde in phosphate buffered saline (PBS)		
Falcon Tube (50 mL)		Fischer Scientific
DMSO		
Disposable gloves, nitrile		
Surflo Winged Infusion Set 21Gx3/4	SV*21BLK	Terumo
Surgical Scissors	14090-09	Fine Science Tools
Standard Pattern Forceps	11001-12	Fine Science Tools
Forceps	11223-20	Fine Science Tools
Isoflurane	CED1360	Penn Veterinary Supply, Inc.

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Falcon Tube (50 mL)		Fischer Scientific
Dimethyl sulfoxide (DMSO)	D2650	Sigma Aldrich
PBS	BP24384	Fisher Scientific
PBS	BP24384	Fisher Scientific
Isoflurane 1-3%	07-893-1389	Patterson Veterinary
Agarose LE	87046	Electron Microscopy Sciences
PBS	BP24384	Fisher Scientific
Silver blue double edge razor blade (Gillette)	884718268996	Amazon
cell culture plate 24 well	View	Sigma-aldrich
DAPI	D1306	Thermo Scientific

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NAME	CATALOG #	VENDOR
Superfrost Plus Microscope Slies	4951PLUS4	Thermo Fisher Scientific
VECTASHIELD® Hardset™ Antifade Mounting Medium	H-1400	Vector Laboratories

Prepare dye and fix

1 Add **100 µl** of DMSO to lyophilized dye (100nmol) and Pipette up and down ~20 times to get 1mM concentration stock.

Dimethyl sulfoxide (DMSO)
by Sigma Aldrich
Catalog #: D2650

2 Mix further using a vortex machine to ensure the dye is evenly distributed in the DMSO

3 Prepare 4% Paraformaldehye in 0.1M PB see here

4 Add 50-100 ul of 1mM dye stock to **50 ml per mouse** of 4% paraformaldehyde in .1M PB

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Set-up hood for perfusion

5 Set up fume hood with:

Peristaltic pump-102R
Watson-Marlow 013.7101.000

Dissection tray
VWR 470006-956

Forceps
Fine Science tools 11223-20

Surgical Scissors
Fine Science tools 14090-09

Perfusion and dissection

Falcon Tube (50 mL)
by Fischer Scientific

6 Perfusion pump preparation

Attach needle:

Surflo winged infusion set 21G 3/4
Terumo TER 3SV-21BLK

to pump tubing and flush with ~ **20 ml** of 1x PBS

PBS
by Fisher Scientific
Catalog #: BP24384

to clear tubing of previous solution

7 Retreive animal per vivarium protocol

8 Anesthetize mouse in 2000ml glass beake

8.1 pour **5 ml** isoflorane down the side of the container.

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8.2 Isoflurane 1-3%
by Patterson Veterinary
Catalog #: 07-893-1389
Place the animal inside the container on a raised platform and cover. (Platform can be square slides box lid or styrafoam from 50ml tube packaging)
(DO NOT allow the liquid isofluorane to come into contact with the animals' skin)

8.3 ⚠

As soon as the animal stops breathing, remove from contain, check for pain response by gently pinching the foot with forceps and make sure no reflexive response and place on dissection tray.
Timing is extremely important. If the animals is over-exposed to the isofluorane than the heart will stop beating and cell death can occur.

9 Begin perfusion

9.1 Lay mouse on its back and place a pin in each front paw, others as needed.

9.2 Use scissors to cut parallel to the sternum, opening the chest cavity and exposeing the heart.

9.3 Insert winged needle into the right ventricle.

9.4 Cut left atrium.

9.5 Start perfusion pump and flush **50 ml 4% PFA** with **100 µl of Dye** at a rate of 7.5ml/min.
(Animal will "twitch" when the fixative circulates through the bloodstream. If no reaction occurs check postion of the needle in the heart.)

9.6 "Stop" perfuison pump when all fixative is flushed through the animal.

9.7 Unpin mouse from dissection tray and decapitate behind ears.
(Place carcass in small biohazard bag for disposal.)

9.8 Remove the fur from the head and cut small slits into the skull by placing scissor tip on eithier side of the opening where the spinal cord connect to the brain.

9.9 Use forceps to peel off skull cap and scope out brain.

9.10 Post-fix brain in **20 ml 4% PFA** **16:00:00** at **4 °C**

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9.11 Rinse 3 times **00:15:00** **00:15:00** **00:15:00** in PBS

PBS
by Fisher Scientific
Catalog #: BP24384

at **Room temperature**

Prep brain for sectioning

10 Prepare **50 ml 4% agarose**

10.1 Add **2.5 g**

Agarose LE
by Electron Microscopy Sciences
Catalog #: 87046

into **50 ml 1x PBS**

PBS
by Fisher Scientific
Catalog #: BP24384

in 500ml glass bottle with cap.
(do not fully tighten cap onto bottle so steam can vent while heating)

10.2 Heat in microwave for **00:01:20** .

Check to see if all agarose is dissolved. If not re-heat at **00:00:10** intervals till completely dissolved.

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10.3 Pour agarose into

Peel-A-Way® Embedding Mold (Square - S22)
polysciences 18646A-1

and orient brain in mold.

10.4 Allow agarose to set about **00:10:00**
(will change from transparent to slighly cloudy and will become firm to the touch.)

11 Prep Vibratome

VT 1200s Vibratome
Leica 1491200S001

with

Silver blue double edge razor blade (Gillette)
by Amazon
Catalog #: 884718268996

11.1 Adjust vibratome settings:

Amplitude 1.00mm
Speed .70mm/s
Feed (thickness) 100um

11.2 Fill buffer tray with **300 ml 1x PBS**

Section brain

12 Section desired region at 50um or 100um.

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12.1 Float sections in

cell culture plate 24 well
by Sigma-aldrich
View

(Single section per well) with each well filled with **500 µl 1x PBS**

(Label plate and wrap with aluminum foil)

DAPI stain

13 DAPI stain

13.1 Add **2.1 µl DAPI stock** to **50 ml 1xPBS**

DAPI
by Thermo Scientific
Catalog #: D1306

13.2 Select desired sections and remove PBS from wells.

13.3 Add **500 µl per well** of DAPI stain to each well for **02:00:00**

13.4 Rinse 2 times **00:05:00** **00:05:00** with **500 µl 1x PBS**

Mount and coverslip with Vecatshield

14 Mount sections on

Superfrost Plus Microscope Slies
by Thermo Fisher Scientific
Catalog #: 4951PLUS4

14.1 Allow sections to dry for at least **02:00:00**

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14.2 Rehydrate slides in **250 ml 1x PBS** for **00:30:00**

14.3 Cover-slip slides with **130 µl vecatshield** and allow to fully dry for **12:00:00**

VECTASHIELD® Hardset™ Antifade Mounting Medium
by Vector Laboratories
Catalog #: H-1400

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