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

Forked from a private protocol



In 2 collections

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Protocol status: Working

We use this protocol and it's working

Created: August 07, 2019

Last Modified: January 20, 2025

Protocol Integer ID: 26635



Materials

MATERIALS

- ☒ Phosphate Buffered Saline **Thermo Fisher Scientific Catalog #28374**
- ☒ 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 **Terumo Catalog #SV*21BLK**
- ☒ Surgical Scissors **Fine Science Tools Catalog #14090-09**
- ☒ Standard Pattern Forceps **Fine Science Tools Catalog #11001-12**
- ☒ Forceps **Fine Science Tools Catalog #11223-20**
- ☒ Isoflurane **Penn Veterinary Supply, Inc. Catalog #CED1360**

STEP MATERIALS

- ☒ Dimethyl sulfoxide (DMSO) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D2650**
- ☒ Falcon Tube (50 mL) **Fischer Scientific**
- ☒ Isoflurane 1-3% **Patterson Veterinary Catalog #07-893-1389**
- ☒ Silver blue double edge razor blade (Gillette) **Amazon Catalog #884718268996**
- ☒ cell culture plate 24 well **Merck MilliporeSigma (Sigma-Aldrich)**
- ☒ Superfrost Plus Microscope Slies **Thermo Fisher Scientific Catalog #4951PLUS4**
- ☒ PBS **Fisher Scientific Catalog #BP24384**
- ☒ Agarose LE **Electron Microscopy Sciences Catalog #87046**
- ☒ PBS **Fisher Scientific Catalog #BP24384**
- ☒ DAPI **Thermo Scientific Catalog #D1306**
- ☒ VECTASHIELD® Hardset™ Antifade Mounting Medium **Vector Laboratories Catalog #H-1400**
- ☒ PBS **Fisher Scientific Catalog #BP24384**



Protocol materials

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Prepare dye and fix

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



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

- 2 Mix further using a vortex machine to ensure the dye is evenly distributed in the DMSO
- 3 Prepare 4% Paraformaldehyde 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

Set-up hood for perfusion

- 5 Set up fume hood with:

Equipment

Dissection tray

NAME

VWR

BRAND

470006-956

SKU

Equipment

Peristaltic pump-102R

NAME

Watson-Marlow

BRAND

013.7101.000

SKU

Equipment

Surgical Scissors

NAME

Fine Science tools

BRAND

14090-09

SKU

<https://www.finescience.com/en-US/Search?searchtext=14090-09>^{LINK}

Equipment

Forceps

NAME

Fine Science tools


BRAND

11223-20

SKU

<https://www.finescience.com/en-US/Products/Forceps-Hemostats/Dumont-Forceps/Dumont-2-Laminectomy-Forceps/11223-20>

LINK

 Falcon Tube (50 mL) **Fischer Scientific**

Perfusion and dissection

6 Perfusion pump preparation

Attach needle:

Equipment

Surflo winged infusion set 21G 3/4

NAME

Terumo

BRAND

TER 3SV-21BLK

SKU

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


 PBS **Fisher Scientific Catalog #BP24384**

to clear tubing of previous solution

7 Retrieve animal per vivarium protocol



8 Anesthetize mouse in 2000ml glass beake

8.1 pour  5 mL isoflorane down the side of the container.

 Isoflurane 1-3% **Patterson Veterinary Catalog #07-893-1389**

8.2 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 μ L 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 either 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


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

 PBS 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 Electron Microscopy Sciences Catalog #87046


into  50 mL 1x PBS

 PBS 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.

10.3 Pour agarose into

Equipment

Peel-A-Way® Embedding Mold (Square - S22) NAME


polysciences

BRAND

18646A-1

SKU

and orient brain in mold.

10.4 Allow agarose to set about  00:10:00

(will change from transparent to slightly cloudy and will become firm to the touch.)

11 Prep Vibratome

Equipment

VT 1200s Vibratome

NAME

Leica

BRAND

1491200S001

SKU

<https://www.leicabiosystems.com/histology-equipment/sliding-and-vibrating-blade-microtomes/vibrating-blade-microtome/leica-vt1200-s/>

LINK



with

 Silver blue double edge razor blade (Gillette) **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.

12.1 Float sections in

 cell culture plate 24 well **Merck MilliporeSigma (Sigma-Aldrich)**

(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 **Thermo Scientific Catalog #D1306**

13.2 Select desired sections and remove PBS from wells.



13.3 Add  500 μ L 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 μ L 1x PBS



Mount and coverslip with Vecatshield

14 Mount sections on

 Superfrost Plus Microscope Slies **Thermo Fisher Scientific Catalog #4951PLUS4**

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

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

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

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