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

CATALOC #

VENDOD

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



MATERIALS

NAME >		CATALOG #	VENDOR V	
Phosphate Buffered Saline		28374	Thermo Fisher Scientific	
Paraformaldehyde fixative: 4% paraformaldehyde in phosphate b	ouffered 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 So	Fischer Scientific	
Dimethyl sulfoxide (DMSO)	D2650	Sigma Alo	Sigma Aldrich	
PBS	BP24384	Fisher Sci	Fisher Scientific	
PBS	BP24384	Fisher Sci	Fisher Scientific	
Isoflurane 1-3%	07-893-1389	Patterson	Veterinary	
Agarose LE	87046	Electron N	Microscopy Sciences	
PBS	BP24384	Fisher Sci	entific	
Silver blue double edge razor blade (Gillette)	884718268996	Amazon		
cell culture plate 24 well	View	Sigma-alo	drich	
DAPI	D1306	Thermo S	cientific	

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

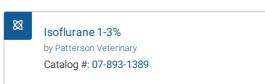
Prepare dye and fix

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



- 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 **30 ml per mouse** of 4% paraformaldehyde in .1M PB

Set-up hood for perfusion Set up fume hood with: Dissection tray Peristaltic pump-102R VWR 470006-956 Watson-Marlow 013.7101.000 Surgical Scissors Forceps Fine Science tools 14090-09 Fine Science tools 11223-20 Perfusion and dissection Falcon Tube (50 mL) by Fischer Scientific 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 88 **PBS** by Fisher Scientific Catalog #: BP24384 to clear tubing of previous solution Retreive animal per vivarium protocol Anesthetize mouse in 2000ml glass beake 8 8.1 pour **5 ml** isoflorane down the side of the container.



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 350 ml 4% PFA with 3100 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.)
- 9.7 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 616:00:00** at **84°C**

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at § Room temperature



- 10 Prepare **50 ml 4% agarose**
- 10.1 Add **□2.5** g



into 30 ml 1x PBS



in 500ml glass bottle with cap.

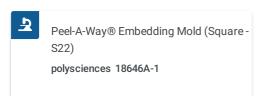
(do not fully tighten cap onto bottle so steam can vent while heating)

10.2 Heat in microwave for \bigcirc **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



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



with



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.

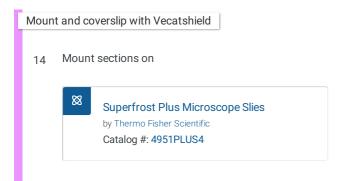
12.1 Float sections in cell culture plate 24 well by Sigma-aldrich View (Single section per well) with each well filled with □500 μl 1x PBS (Label plate and wrap with aluminum foil)

DAPI stain

- 13 DAPI stain
- 13.1 Add **□2.1** µl DAPI stock to **□50** ml 1xPBS



- 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 \bigcirc 00:05:00 \bigcirc 00:05:00 with \square 500 μ l 1x PBS



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 \blacksquare 130 μ l vecatshield and allow to fully dry for \bigcirc 12:00:00
 - VECTASHIELD® Hardset™ Antifade Mounting Medium by Vector Laboratories Catalog #: H-1400

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