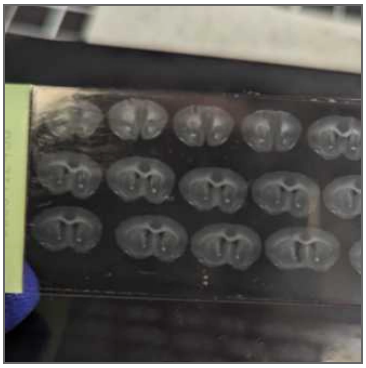

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Protocol status: Working
We use this protocol and it's working

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Mounting and Coverslipping Mouse Brain Sections

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ABSTRACT

This protocol details mounting thin sliced mouse brain tissue sections onto charged slides in a uniform orientation that does not create wrinkles or artifacts that might interfere with imaging, and then how to apply a coverslip. After mounting and coverslipping, slides will be ready for imaging.

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Keywords: Histology, Immunohistochemistry, Coverslip, Tissue Mounting

MATERIALS

10xPBS **Ambion** **Catalog #**AM9624

Milli-Q Water **Contributed by users**

Triton X-100 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T8787-50ML

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

Vectashield hardset anti-fade mounting medium with dapi **Vector Laboratories** **Catalog #**H-1200-10

ProLong glass antifade mountant **Thermo Fisher Scientific** **Catalog #**P36980

	Materials	Product number
	Fine tip paintbrush	Amazon, PBR-01
	Fisherbrand Tissue Path Superfrost Plus Gold Slides	Fisher Scientific, 5158848
	Petri dish	Thermofisher Scientific, 263991
	Aluminum foil	Amazon, B074NB5CDZ
	Dissection scope	Grainger, 41111709
	Well plate	Costar, 3548
	Squeeze bottle	Amazon, B00WTHLR18
	Cover glass signature series	Richard-Allan Scientific, 12455S
	Disposable transfer pipet	Falcon, 52947-970

Recipes:

1L 1xPBS:

Combine the following reagents into a container with a stir bar. Mix well on a stir plate at high speed (300 RPM or higher) for 00:02:00 or until solution is mixed. Store at Room temperature for 1 month.

	Reagent	Volume
	Milli-Q water	900mL
	10xPBS	100mL

1L 1xPBS & Triton X-100 0.2% :

Put 1xPBS into a container with a stir bar and slowly pipet Triton X-100 in. Mix well on a stir plate at high speed (300 RPM or higher) for 00:15:00 to ensure Triton X-100 goes into solution. Store at Room temperature for 1 month.

	Reagent	Volume
	1xPBS	998mL
	Triton X-100	2mL

SAFETY WARNINGS

Wear gloves and appropriate PPE as mouse brain tissue may contain trace amounts of Azide and Paraformaldehyde.

BEFORE START INSTRUCTIONS

Mouse brain slices must first be fixed in 4% PFA and stained with desired dyes or antibodies, and typically stored in a well plate prior to mounting. Refer to protocols for sectioning and staining tissue to prepare tissue prior to mounting: Sectioning Mouse Brain with Sliding Microtome and Immunohistochemistry (IHC) Staining Mouse Brain Sections.

Mounting

1h

1 Prepare a petri dish filled with 1xPBS & Triton X-100 0.2%.

2 Place a drop of 1xPBS onto a 1"x3" slide.

3 Using a small paintbrush, lift the first tissue section to be mounted out of the storage well plate and place section into the petri dish so that it lays flat close to the surface of the 1xPBS & Triton X-100 0.2% solution.

4 Using paintbrush, lift the tissue section out of the petri dish and place it onto the slide. Arrange tissue section with paintbrush to ensure section is properly aligned, flat, and wrinkle-free with small paintbrush. This can usually be done by eye, but a dissection scope may be used if desired at times when it is difficult to see fine anatomical detail in the slices macroscopically (just by eye).

5 Repeat steps 2 -4 until all sections have been mounted onto the slides.

Note

Mount sections onto each slide in one to three rows, so that they all have the same left/right orientation. Often the cerebral cortex will be shallowly notched during slicing on the left or right side to indicate left/right orientation, it will be helpful to refer to this.

6 Protect slides from light and allow slides to dry at Room temperature for a minimum of 01:00:00 1h or until adhered to the slide.

Note

Slides can be protected from light by placing a sheet of aluminum foil or a box lid over the slides, or any other equivalent method of covering slides to shield from light.

7 Once sections are dry, rinse off 1xPBS residue by briefly rinsing the slide with Milli-Q water.

8 Blot off any residual Milli-Q water by pressing the edge of the slide onto a kimwipe.

Note

Proceed to step 9 immediately -- slides should be coverslipped same day as mounting.

Coverslipping

9 Choose requested mounting medium:

	Frequently requested mounting media	Notes
	Vectashield hardset anti-fade mounting medium, Vector Laboratories (H-1400-10)	
	Vectashield hardset anti-fade mounting medium with dapi, Vector Laboratories (H-1500-10)	Generally not requested if dapi was already used as a secondary stain. Staining with dapi as a secondary stain provides stronger staining than vecta shield with dapi.
	ProLong Glass Antifade Mountant (P36980)	Good for minimizing optical refraction and preserving fluorescent signal

10 Coverslip slide with mounted tissue:

10.1 Place a few drops of chosen mounting medium onto the slide to be coverslipped, making sure there are no bubbles. If mounting medium bottle does not have dropper attachment, use a pipette to place drops on slide.

10.2 Start with only one edge of the coverslip touching the corresponding edge of the slide, and slowly lay down the coverslip.

11 If re-coverslipping is required due to air bubbles overlapping onto tissue sections or other artifacts, soak slide in 1xPBS in a petri dish until coverslip comes off easily and repeat step 10. Do not use force to pull off a stuck coverslip; soak the slide longer.

Correctly coverslipped slide with no air bubbles overlapping tissue sections.

12 Allow slides to dry Overnight at Room temperature, protecting from light by covering slides with a sheet of aluminum foil, a box top, or other material that will shield slides from light.

13 Store slides at 4 °C protected from light until ready for imaging. Slides can be stored for several months.