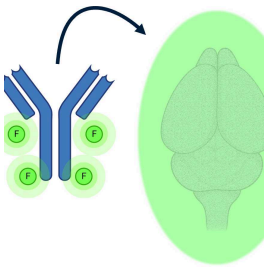
Jun 06, 2023 Version 1

Immunolabeling of a Whole Mouse Brain V.1



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

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Keywords: immunolabeling, whole brain, antibody, delipidated brain sample, fluorescent protein, viral labeling of target neuron population, encoded fluorescent protein, whole mouse brain, whole brain sample, target neuron population, antibody labeling, accessible to antibody labeling, mice, antibody, brain, immunolabeling, fluorophore, concentration of fluorophore, organic solvent delipidation step, delipidation, signal from endogenous fp

Funders Acknowledgements:
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Abstract

Our mice have sparse viral labeling of target neuron populations with genetically encoded fluorescent proteins (FPs). Delipidation ensures that whole brain samples become optically clear and more accessible to antibody labeling. However, the signal from endogenous FPs is often dimmed or quenched during organic solvent delipidation steps. Tissue expansion also dilutes the concentration of fluorophores. To amplify signals of FPs for high resolution, multi-scale imaging, we immunolabel delipidated brain samples.

Materials

PBS - Phosphate-Buffered Saline (10X) pH 7.4 **Invitrogen - Thermo Fisher Catalog #**AM9625

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

Tween 20 **Catalog #**P1379

5% Sodium Azide **Fisher Scientific Catalog #**71448-16

Equipment

VWR® Standard Orbital Shaker, Model 3500 — NAME

Shaker — TYPE

VWR — BRAND

89032-092 — SKU

https://us.vwr.com/store/product/4901055/vwr-standard-orbital-shaker-model-3500 — LINK

Equipment

WHEATON® Liquid Scintillation Vials, Caps Attached to Vials, Glass, Polyethylene Cone, 22-400, 20 mL

NAME

Vial

TYPE

Wheaton

BRAND

DWK986546

SKU

https://www.dwk.com/na/wheaton-liquid-scintillation-vials-caps-attached-to-vials-glass-polyethylene-cone-22-400-20-ml-986546

LINK

20 mL Glass Vial with Polyethylene cone Caps

SPECIFICATIONS

Equipment

WHEATON® Shorty Vials clear with PTFE faced rubber lined cap

NAME

vial

TYPE

WHEATON

BRAND

DWKW224607

SKU

https://www.sigmaaldrich.com/US/en/product/aldrich/dwkw224607

LINK

Equipment

Nutating Mixer	NAME
Mixer	TYPE
Fisherbrand	BRAND
88-861-043	SKU
https://www.fishersci.com/shop/products/nutating-mixers-variable-speed/88861043	LINK
16.3 × 11.5 × 10.7 in.(415 × 293 × 273 mm)	SPECIFICATIONS

RECIPES

PTxw: 0.05% Tween 20, 0.1% Triton X-100, 0.04% NaN_3 in PBS

Combine the following reagents and stir at Room temperature until fully dissolved, store at 4 °C.

	Reagent	Amount
	Milli-Q Water	up to 500 mL
	10X PBS	50 mL
	Triton X-100	500 uL
	Tween 20	250 uL
	5% Sodium azide	4 mL
	Total	**500 mL**

Troubleshooting

Before start

If performing the **Whole Mouse Brain Delipidation, Immunolabeling, and Expansion Microscopy** protocol, start with a brain that has been delipidated using the **Tetrahydrofuran and Dichloromethane Delipidation of a Whole Mouse Brain** and **Aqueous (SBiP) Delipidation of a Whole Mouse Brain** protocols.

Immunolabeling of a Whole Mouse Brain

4w 3d

1 Start with a whole mouse brain perfused with 4% PFA, post-fixed, and stored in PBS in a 20 mL vial.

2 Using a 20 mL glass vial, wash the brain with PTxw, rotating at Room temperature Overnight

3 Transfer the brain to a small 4 mL vial containing diluted primary antibody in ~ 4 mL PTxw and incubate with rocking, a nutator or shaker, at Room temperature for 11 days. 1w 4d

- 10 µg/brain for primary antibody
- 20 µg/brain for primary conjugated antibody

4 Transfer the brain to a 20 mL glass vial and wash with PTxw, rocking on a nutator or shaker at Room temperature, and replacing solution for each step: 3d 11h

- PTxw for 01:00:00
- PTxw for 02:00:00
- PTxw for 04:00:00
- PTxw Overnight
- PTxw for 24:00:00
- PTxw for 24:00:00
- PTxw for 24:00:00 +

5 Transfer the brain to a small 4 mL vial containing diluted secondary antibody in ~ 4 mL PTxw and incubate with rocking at Room temperature for 11 days.

- 20 µg/brain for secondary antibody

6 Transfer brain to a 20 mL glass vial and wash with PTxw, rocking at Room temperature, and replacing solution for each step: 3d 11h

- PTxw for 01:00:00
- PTxw for 02:00:00
- PTxw for 04:00:00

- PTxw Overnight
- PTxw for 24:00:00
- PTxw for 24:00:00
- PTxw for 24:00:00 +

7 Wash the brain with 1X PBS, rocking at Room temperature Overnight . 16h