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

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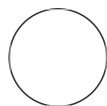
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🌐 Immunolabeling of a Whole Mouse Brain

Andrew Recknagel¹, Kevin Cao², Molly Logsdon², Judith Baka², Naveen Ouellette², Jayaram Chandrashekar²

¹Janelia Farm; ²Allen Institute for Neural Dynamics



Naveen Ouellette

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 Contributed by users 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 x 11.5 x 10.7 in.(415 x 293 x 273 mm)	SPECIFICATIONS
	

RECIPES

PTxw: 0.05% Tween 20, 0.1% Triton X-100, 0.04% NaN₃ 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

BEFORE START INSTRUCTIONS

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  1w 4d and incubate with rocking, a nutator or shaker, at  Room temperature for 11 days.
 - 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  3d 11h

🌡️ Room temperature , and replacing solution for each step:

- 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 3d 11h replacing solution for each step:

- 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