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Aqueous (SBiP) Delipidation of a Whole Mouse Brain V.1

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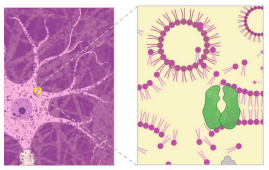

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Protocol status: Working
We use this protocol and it's working

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Keywords: delipidation, aqueous delipidation, clearing, brain delipidation, lipid removal via phase separation, whole mouse brain aqueous strategy, organic delipidation, delipidation, lipid removal, aqueous biphasic buffer, sbip, phosphate buffer, solvent, lipid, aqueous strategy, forming micelle, phase separation, antibody, whole mouse brain, residual membrane, b1n buffer, antibody labeling, brain, detergent, buffer

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Abstract

Aqueous strategies for whole-brain delipidation involve lipid removal via phase separation and detergent washes. SBiP is an aqueous biphasic buffer that extracts lipids at polar-nonpolar solvent interfaces. The brain is then washed with the detergent-based B1n buffer, which further disrupts residual membranes, forming micelles that can be washed out. When paired with organic delipidation, these steps can return a solvent-shrunken brain to normal size in phosphate buffer, suitable for post-delipidation antibody labeling.

Guidelines

It is recommended to etch identification information on the glass vial. Ink or label adhesive may dissolve if exposed to organic solvents.

Materials

Sodium Dodecyl Sulfate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**74225

Sodium phosphate dibasic **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**7558-79-4

Sodium phosphate monobasic monohydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S9638

2-methyl-2-butanol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**152463

2-propanol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**278475

Glycine **Fisher Scientific** **Catalog #**BP381-500

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

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

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

10N NaOH **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**SX0607N-6

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

Fisherbrand™ Multi-Purpose Tube Rotator	NAME
Carousel	TYPE
FisherBrand	BRAND
88-861-049	SKU
https://www.fishersci.com/shop/products/multi-purpose-tube-rotators/88861049	LINK

RECIPES

10 mM Phosphate Buffer pH 8.3

Combine the following reagents, adjust pH to 8.3.

	Reagent	Amount	Final Concentration
	1M Phosphate Buffer	5 mL	10 mM
	Milli-Q water	495 mL	

SBiP Solution: 0.08% SDS, 16% 2-Methyl-2-Butanol, 8% 2-Propanol, in H_2O

Combine the following reagents on ice. Use a fume hood when adding 2-methyl-2-butanol and 2-propanol. Mix On ice until solution is uniform and clear. Store immediately at 4 °C until ready for use. Use each batch within a month for best effect.

	Reagent	Volume
	Milli-Q water (ice cold)	350 mL
	50mM Na2HPO4	2 mL
	4% SDS (in H2O, pH 7.4)	10 mL
	2-Methyl-2-butanol	80 mL
	2-Propanol	40 mL
	Total	**482 mL**

B1n Buffer: 0.1% Triton X-100, 2% Glycine, 0.02% NaN_3 in H_2O

Combine the following reagents and stir at Room temperature until fully dissolved. Store for a few months at Room temperature.

	A	B
	Milli-Q Water	up to 500 mL
	Triton X-100	500 uL

	A	B
	Glycine	10 g
	10N NaOH	50 uL
	5% Sodium azide	2 mL
	Total	**500 mL**

Troubleshooting

Safety warnings

2-Methyl-2-butanol and 2-propanol are corrosive and flammable. Perform the steps that involve these reagents under the fume hood. Wear a lab coat, safety goggles or glasses, and gloves.

Before start

If performing this delipidation as part of the **Whole Mouse Brain Delipidation, Immunolabeling, and Expansion Microscopy** protocol, first delipidate the whole mouse brain with organic solvents using the **THF and DCM Delipidation of a Whole Mouse Brain** protocol.

SBiP Delipidation

5d 12h

1 **Delipidate with aqueous SBiP solution**

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

2 Use a 20 mL vial for processing an adult mouse brain. All steps in this section are carried out on a carousel rotator 10 rpm, Room temperature .

4d 9h

Replace solution in vial with 20 mL SBiP for each of the following steps:

- SBiP for 03:00:00
- SBiP for 06:00:00
- SBiP Overnight
- SBiP for 24:00:00
- SBiP for 24:00:00
- SBiP for 24:00:00
- SBiP for 24:00:00

Note

It is important that the SBiP solution is mixed thoroughly during the aqueous steps in order to effectively delipidate the brain. When it is thoroughly mixed, the solution should look uniformly turbid at Room temperature .

Safety information

2-Methyl-2-butanol and 2-propanol are corrosive and flammable. Perform the steps that involve these reagents under the fume hood. Wear a lab coat, safety goggles or glasses, and gloves.

3 Wash the brain with 20 mL B1n buffer at Room temperature Overnight .

4 Replace B1n buffer to complete ~ 24:00:00 wash. 1d

5 Wash the brain with 1X PBS, rotating at Room temperature for the following steps: 3h

- PBS for 01:00:00
- PBS for 02:00:00 +

6 Aqueous (SBiP) delipidation complete. Store in 1X PBS 0.05% Azide for up to 6 months.