

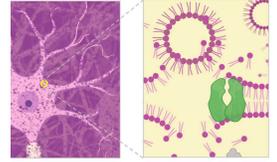
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Version 2

Aqueous (SBIp) Delipidation of a Whole Mouse Brain V.2

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Protocol status: Working

We use this protocol and it's working

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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 H₂O

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 Na ₂ HPO ₄	2 mL
4% SDS (in H ₂ O, 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% NaN₃ in H₂O

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.

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

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.