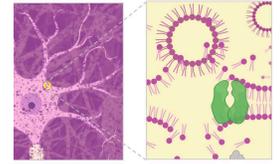


Apr 23, 2025

Version 2

Tetrahydrofuran and Dichloromethane Delipidation of a Whole Mouse Brain V.2



DOI

<https://dx.doi.org/10.17504/protocols.io.36wgqj1kxvk5/v2>

Andrew Recknagel¹, Kevin Cao², Judith Baka², Naveen Ouellette², Molly Logsdon², Jayaram Chandrashekar², Rajvi Javeri²

¹Janelia Research Campus; ²Allen Institute for Neural Dynamics

Allen Institute for Neural...



Naveen Ouellette

Allen Institute

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.36wgqj1kxvk5/v2>

Protocol Citation: Andrew Recknagel, Kevin Cao, Judith Baka, Naveen Ouellette, Molly Logsdon, Jayaram Chandrashekar, Rajvi Javeri 2025. Tetrahydrofuran and Dichloromethane Delipidation of a Whole Mouse Brain. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.36wggj1kxvk5/v2> Version created by **Hannah Belski**

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: April 08, 2025

Last Modified: April 24, 2025

Protocol Integer ID: 126390

Keywords: delipidation, tissue clearing, clearing, whole mouse brain organic solvent strategy, dichloromethane as the main delipidation solvent, dehydration solvent, brain delipidation, dichloromethane delipidation, main delipidation solvent, delipidated whole brain, extensive dehydration gradient, aqueous delipidation step, dichloromethane, immiscible solvent, cell membrane, tetrahydrofuran, tissue shrinkage, miscible solvent, solvent, delipidation, whole mouse brain, brain

Funders Acknowledgements:

Allen Institute

Abstract

Organic solvent strategies for whole-brain delipidation involve dehydrating the tissue in a water-miscible solvent followed by washes with a water-immiscible solvent. Tissue shrinkage will occur, but when paired an aqueous delipidation step and expansion chemistry becomes negligible. Additionally, an extensive dehydration gradient is used to preserve tissue morphology. We use tetrahydrofuran (THF) as the dehydration solvent, which helps permeabilize cell membranes. THF is then transitioned into dichloromethane as the main delipidation solvent. This protocol produces a delipidated whole brain that can be further processed for expansion or index-matched for immediate imaging.

Guidelines

THF and DCM will damage many types of plastics. Perform delipidation in a glass vial with teflon screw-top cap. Use glass pipets to transfer reagents.

For thorough delipidation, the tissue needs to be dehydrated as much as possible before DCM is added. Use anhydrous tetrahydrofuran (THF) for the 100% THF steps. Store THF and DCM under inert atmosphere. If possible, purchase THF in small volume (100 mL) septa-sealed bottles.

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

Materials

 Dichloromethane Merck MilliporeSigma (Sigma-Aldrich) Catalog #320269

 Tetrahydrofuran Merck MilliporeSigma (Sigma-Aldrich) Catalog #186562

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

PYREX® Reusable Serological Pipettes, Glass, Corning, 10 mL NAME

pipet TYPE

PYREX®, Corning BRAND

7085-10 SKU

<https://us.vwr.com/store/product/4760135/pyrex-reusable-serological-pipets-glass-corning> 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



Troubleshooting

Safety warnings

! Tetrahydrofuran:

Tetrahydrofuran (THF) is toxic, carcinogenic, and highly flammable. Wear lab coat, safety goggles or glasses, and chemical resistant gloves (7.8 MIL). Perform the steps that involve these reagents under the fume hood. If these solvents contact your gloves, remove immediately and don new gloves.

When exposed to air, THF may form explosive peroxides if concentrated by distillation or evaporation.

Test for peroxide formation or discard after 1 year.

Dispose of THF in a hazardous waste stream.

Dichloromethane:

Dichloromethane (DCM) is toxic and carcinogenic. Wear lab coat, safety goggles or glasses, and chemical resistant gloves (7.8 MIL). Perform the steps that involve DCM under the fume hood. If these solvents contact your gloves, remove immediately and don new gloves.

Dispose of DCM in a hazardous waste stream.

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

Start with a whole mouse brain perfused with 4% PFA, post-fixed and washed in PBS.

THF and DCM Delipidation of a Whole Mouse Brain

1w

- 1 Start with a whole mouse brain perfused with 4% PFA and post-fixed for 03:00:00 to 06:00:00 at Room temperature and stored at 4 °C for ~ 12:00:00 or Overnight , then washed in 1X PBS.

Note

Perform delipidation in a glass vial with teflon screw-top cap. Use glass pipets to transfer reagents.

Note

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

Note

For each step, use ~ 20 mL or more of solution which should fill to the top of an appropriate sized vial. We use a 20 mL glass scintillation vial.

2 Dehydrate through H₂O → THF series

2d

Wash with tetrahydrofuran (THF) solution (diluted in water) for each whole brain, rotating on a nutator at 4 °C for each step:

- 30% THF for 04:00:00 + or Overnight
- 50% THF for 04:00:00 + or Overnight
- 70% THF for 04:00:00 + or Overnight
- 80% THF for 04:00:00 + or Overnight
- 90% THF for 04:00:00 + or Overnight
- 95% THF for 04:00:00 + or Overnight



Safety information

THF is toxic, carcinogenic, and highly flammable. Wear lab coat, safety goggles or glasses, and chemical resistant gloves (7.8 MIL). Perform the steps that involve these reagents under the fume hood. If these solvents contact your gloves, remove immediately and don new gloves.

When exposed to air, THF may form explosive peroxides if concentrated by distillation or evaporation.

Test for peroxide formation or discard after 1 year.

Dispose of THF in a hazardous waste stream.

3 Complete Dehydration Steps

22h

Wash with 100% THF, rotating on a nutator at  4 °C for each step:

- 100% THF for  04:00:00 + or  Overnight
- 100% THF  Overnight
- 100% THF for  02:00:00 + or  Overnight

Note

For thorough delipidation, the tissue needs to be dehydrated as much as possible before DCM is added. Use anhydrous tetrahydrofuran (THF) for the 100% THF steps. Store THF under inert atmosphere. If possible, purchase THF in small volume (100 mL) septa-sealed bottles.

4 Dichloromethane Delipidation

5h

Delipidate in 100% dichloromethane (DCM), rotating on a nutator at  4 °C for each step:

- 100% DCM for  01:00:00
- 100% DCM for  02:00:00
- 100% DCM for  02:00:00

Note

Store DCM under inert atmosphere.

Safety information

DCM is toxic and carcinogenic. Wear lab coat, safety goggles or glasses, and chemical resistant gloves (7.8 MIL). Perform the steps that involve DCM under the fume hood. If these solvents contact your gloves, remove immediately and don new gloves.

Dispose of DCM in a hazardous waste stream.

- 4.1 At the end of DCM delipidation, the brain should sink to the bottom of the vial. If not, let the brain incubate longer and perform additional washes until it sinks.

Note

Pause Point:

If the brain is being delipidated using the Whole Mouse Brain Delipidation, Immunolabeling, and ExM protocol, proceed with steps below. If the brain is being delipidated for a DISCO based cleared method, then move forward with appropriate index matching step.

- 5 Wash with 100% THF, rotating on a nutator at  4 °C for each step:

22h

- 100% THF for  04:00:00 + or  Overnight
- 100% THF  Overnight
- 100% THF for  02:00:00 + or  Overnight

Safety information

See THF safety information from step 2 above.

- 6 **Rehydrate through THF → H₂O series**

1d 18h

Wash with tetrahydrofuran (THF) solution (diluted in water) for each whole brain, rotating on a nutator at  4 °C for each step:

- 95% THF for  02:00:00 + or  Overnight
- 90% THF for  02:00:00 + or  Overnight

- 80% THF for  02:00:00 + or  Overnight
- 70% THF for  02:00:00 + or  Overnight
- 50% THF for  02:00:00 + or  Overnight
- 30% THF for  02:00:00 + or  Overnight
- 10mM Phosphate Buffer pH 8.3  02:00:00 + or  Overnight

Safety information

THF is toxic, carcinogenic, and highly flammable. Wear lab coat, safety goggles or glasses, and chemical resistant gloves (7.8 MIL). Perform the steps that involve these reagents under the fume hood. If these solvents contact your gloves, remove immediately and don new gloves.

When exposed to air, THF may form explosive peroxides if concentrated by distillation or evaporation.

Test for peroxide formation or discard after 1 year.

Dispose of THF in a hazardous waste stream.

- 7 Wash with 1X PBS, rotating at  4 °C  Overnight
- 8 Organic solvent (THF/DCM) delipidation complete. Store brains in 1X PBS 0.05% Azide for up to 6 months.