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Whole Mouse Brain Delipidation - Dichloromethane

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the delipidation of a mouse brain specimen using a modified iDISCO protocol. The brain is dehydrated with methanol and then cleared of lipids with dichloromethane to prepare for refractive index matching (see protocol **Refractive Index Matching - Ethyl Cinnamate**). This will allow for viewing and imaging of internal brain structures with a light sheet microscope. This technique does not preserve endogenous fluorescence in the brain tissue, but we have used it successfully for probe placement validation.

Materials

Reagents:

 Methanol **P212121 Catalog #PA-33900HPLCCS4L**

 Dichloromethane **Bio Basic Inc. Catalog #DC3600.SIZE.1L**

 Milli-Q water **Contributed by users**

Materials	Product number
Aluminum foil	Amazon, B074NB5CDZ
20ml glass scintillation vials	Millipore Sigma, Z376817-1PAK
Serological pipets, plastic	Fisher Scientific, 13-678-11E
Serological pipets, glass	Corning, 7077-10N
Serological pipet filler	Thermofisher, 9501

Safety warnings

- ! Personal Protective Equipment (PPE) should be used at all times while operating this protocol. If you are unsure what PPE you should be using, see your immediate supervisor.

Have appropriate Methanol and Dichloromethane waste containers.

- **Dichloromethane**

Inhalation –can cause coughing, wheezing, and/or shortness of breath. Higher levels of dichloromethane inhalation can lead to headaches, mental confusion, nausea, vomiting, dizziness, and fatigue.

Skin Exposure –Redness and irritation may occur if skin comes in contact with liquid dichloromethane and, if it remains on the skin for an extended period of time, it may lead to skin burns.

Eye Exposure –Contact with the eyes can cause severe irritation and possible chemical burns to the eyes.

Safety Precautions When Handling Dichloromethane

When handling dichloromethane in the workplace, use the following safety precautions:

- Wear protective clothing. Footwear should cover the entire foot.
- Always wear PPE such as chemical splash goggles and safety gloves.
- Work in a well-ventilated area (preferably in an environment with a fume extraction system).

- **Methanol**

When handling methanol, it is best to avoid direct exposure as much as possible.

As such, it is imperative that safety gear be worn, especially those that cover the face, eyes, and skin. If working where methanol vapors are present, proper ventilation is imperative for safety.

Should methanol come into direct contact with the skin, remove any contaminated clothing and wash the affected area with soap and water for 15 minutes. If methanol comes into contact with the eyes, flush immediately with tepid water for 15 minutes and then seek qualified medical help.

Before start

The mouse brain used for this protocol should be fixed in 4% PFA prior to the start of this protocol. See **Mouse Cardiac Perfusion Fixation and Brain Collection V.5** for further details.



Day 1: Dehydrate the PFA-fixed mouse brain in a step gradient of Methanol

1 Prepare 10mL of the following Methanol concentrations:

- 20% Methanol in Milli-Q Water
- 40% Methanol in Milli-Q Water
- 60% Methanol in Milli-Q Water
- 80% Methanol in Milli-Q Water

Safety information

Methanol is highly flammable and toxic. Dispose of any methanol waste in an appropriate waste container.

When handling methanol, it is best to avoid direct exposure as much as possible.

As such, it is imperative that safety gear be worn, especially those that cover the face, eyes, and skin. If working where methanol vapors are present, proper ventilation is imperative for safety.

Should methanol come into direct contact with the skin, remove any contaminated clothing and wash the affected area with soap and water for 15 minutes. If methanol comes into contact with the eyes, flush immediately with tepid water for 15 minutes and then seek qualified medical help.

2 Place whole mouse brain specimen (must be previously fixed in 4% PFA) in 20mL glass vial. Vial should be covered in foil to protect from light and remain covered throughout the rest of the protocol.




Example of how the glass vial should be covered with aluminum foil to protect from light.

3 Dehydrate through H₂O → Methanol series

4h

Wash brain specimen with Methanol solution (diluted in water) for each step using the solutions prepared in step 1. Use a serological pipet to add and remove Methanol solution from the 20ml glass scintillation vial containing the brain specimen for each step.

- 20% Methanol for  01:00:00
- 40% Methanol for  01:00:00
- 60% Methanol for  01:00:00
- 80% Methanol for  01:00:00

- 4 Remove the Methanol solution used in the previous step from the glass vial containing the mouse brain specimen and dispose of it in an appropriate waste container. Measure 100% Methanol into glass vial containing the mouse brain for  01:00:00 .

1h

- 5 Repeat step 7 for a second 100% Methanol  01:00:00 wash.

1h

- 6 Prepare 10mL of 33% Methanol and 66% Dichloromethane solution using 3.33mL Methanol and 6.66mL Dichloromethane. While a plastic serological pipet is adequate for dispensing



Methanol, a glass serological pipet should be used to dispense Dichloromethane.

Safety information

▪ Dichloromethane

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Skin Exposure –Redness and irritation may occur if skin comes in contact with liquid dichloromethane and, if it remains on the skin for an extended period of time, it may lead to skin burns.

Eye Exposure –Contact with the eyes can cause severe irritation and possible chemical burns to the eyes.

Safety Precautions When Handling Dichloromethane

When handling dichloromethane in the workplace, use the following safety precautions:

- Wear protective clothing. Footwear should cover the entire foot.
- Always wear PPE such as chemical splash goggles and safety gloves.
- Work in a well-ventilated area (preferably in an environment with a fume extraction system).

6.1 Leave the whole mouse brain in the 33% Methanol and 66% Dichloromethane solution

Overnight .



Day 2: Delipidation with Dichloromethane

40m

7 Remove 33% Methanol and 66% Dichloromethane solution from the glass scintillation vial using a glass serological pipet.

8 Using a glass serological pipet, measure 10mL of 100% Dichloromethane into the glass scintillation vial for a 00:20:00 wash.

20m

9 Repeat step 8 for a second 100% Dichloromethane 00:20:00 wash.

20m

10 Brain should immediately be index matched with ethyl cinnamate following the second 100% Dichloromethane wash in step 9. See protocol **Refractive Index Matching - Ethyl Cinnamate** for instructions.



Protocol references

<https://link.springer.com/content/pdf/10.1038/s41598-019-47336-9.pdf>