Feb 06, 2025

Refractive Index Matching - Ethyl Cinnamate

DOI
dx.doi.org/10.17504/protocols.io.n2bvj8k4bgk5/v1

Holly Myers[1], Daphne Toglia[2]

[1]Allen Institute for Neural Dynamics; [2]Boston University

Allen Institute for Neural D…

Daphne Toglia
Boston University , Allen Institute for Neural Dynamics

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.n2bvj8k4bgk5/v1

Protocol Citation: Holly Myers, Daphne Toglia 2025. Refractive Index Matching - Ethyl Cinnamate. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.n2bvj8k4bgk5/v1**

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

Created: May 22, 2023

Last Modified: February 06, 2025

Protocol Integer ID: 82287

Keywords: Refractive index matching, Whole mouse brain delipidation, SmartSPIM imaging

Abstract

This protocol details index matching for delipidated whole mouse brain samples in ethyl cinnamate solution to prepare for lightsheet imaging. Upon completion of this protocol, the delipidated mouse brain should be a light amber color and appear translucent when immersed in ethyl cinnamate. This index matching protocol may be paired with a modified iDISCO delipidation protocol: **Whole Mouse Brain Delipidation - Dichloromethane**. Ethyl cinnamate is an inexpensive, effective, and lower-toxicity index matching option that may be used in place of index matching reagents typically paired with this delipidation protocol (Hildebrand et al, 2019).

Materials

Reagents:

Ethyl cinnamate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**112372

	Materials	Product number
	20mL glass scintillation vial	Millipore Sigma, Z376817-1PAK
	Serological pipet	Fisher Scientific, 13-678-11E
	Aluminum foil	Amazon, B074NB5CDZ
	Spatula	Cole Parmer, UX-06287-07
	Petri dish	ThermoFisher, 150460

Safety warnings

Wear gloves at all times during this protocol.

Before start

This protocol is designed to index match a delipidated whole mouse brain specimen. For delipidation methodology, reference protocol **Whole Mouse Brain Delipidation - Dichloromethane**.

Index Matching with Ethyl Cinnamate

3d

1 Ethyl Cinnamate is typically stored at 4 °C . Thaw bottle at Room temperature for 04:00:00 ahead of use, or until the reagent is no longer frozen. 4h

2 Using a serological pipet, measure 10mL of Ethyl Cinnamate into a 20mL glass scintillation vial. Place the delipidated mouse brain specimen into the vial of Ethyl Cinnamate using a spatula.

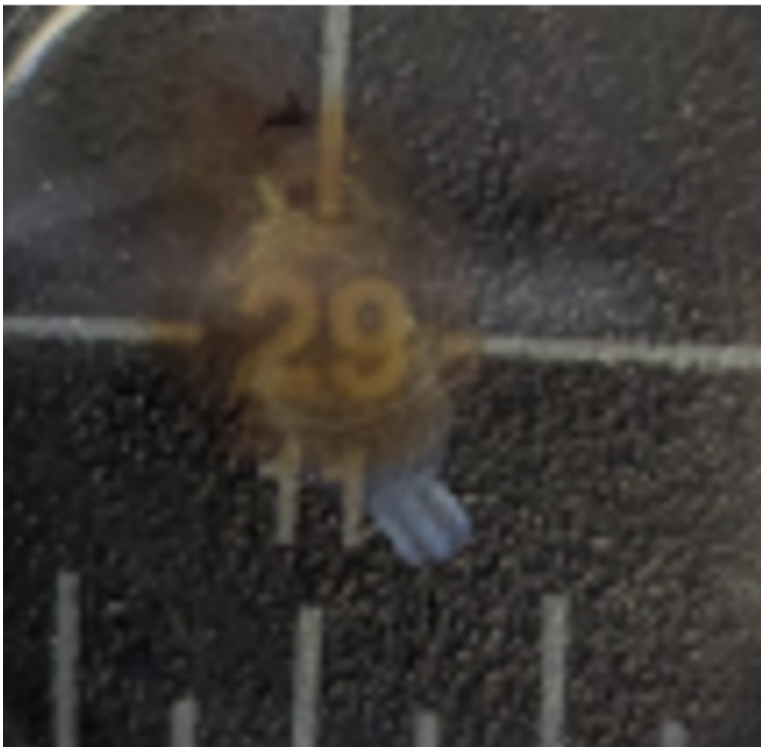
3 Protect the vial containing the brain submerged in Ethyl Cinnamate from light by covering the vial with aluminum foil or placing the vial in a box. Leave for 72:00:00 at Room temperature . 3d

Assessing for Successful Index Matching

3d

4 Assess the brain specimen for successful index matching. It may be helpful to temporarily transfer the brain to a clear petri dish and completely immerse in Ethyl Cinnamate for this step, using a spatula to gently manipulate the brain in the dish if necessary.

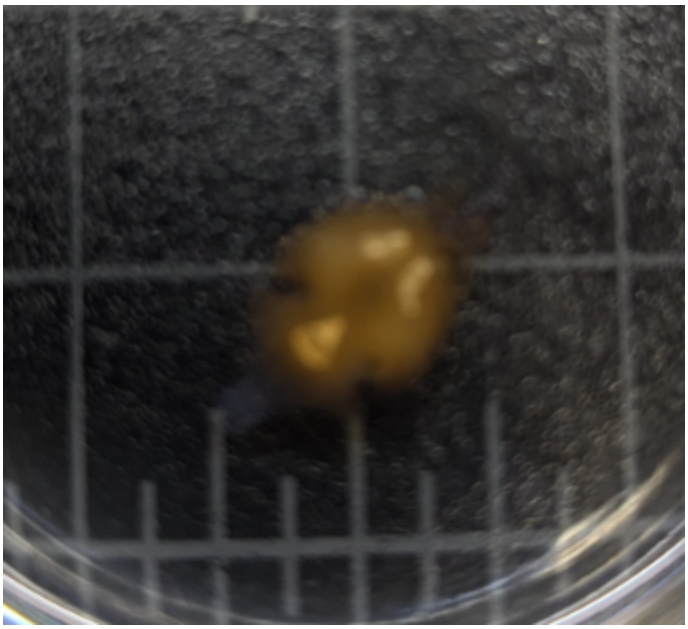
Successful index matching:
When totally immersed in Ethyl Cinnamate, the brain should have an amber color and appear transparent, without visibly opaque interior structures. It should be possible to view numbers, letters, or fine lines on a grid through the brain without warping.



Example of a brain successfully index matched with ethyl cinnamate. Note that when immersed in a dish of ethyl cinnamate, it is possible to view the gridlines and the number "29" through the brain. This brain is ready to be imaged.

Unsuccessful index matching:

Parts of the brain around the cortex may appear transparent, while the deeper structures may have an opaque appearance. It will be difficult to view letters, numbers, or other images through the brain.



Example of a brain not successfully index matched. Note that brain is still opaque and you cannot view any gridlines or numbers through this brain when it is immersed in ethyl cinnamate. This brain should be returned to ethyl cinnamate for longer index matching.

4.1 If the brain appears successfully index matched, it is now ready for light sheet imaging. After imaging, the brain may be stored in Ethyl Cinnamate for several months at Room temperature . 1d

If the specimen isn't properly index matched, consider changing the Ethyl Cinnamate and leaving it for another 24:00:00 at Room temperature before imaging. The time required for clearing and dehydrating is dependent on the size of the specimen -- the larger the specimen, the longer it should be washed in each reagent in order to allow the reagent to fully penetrate the tissue.

Protocol references

Scalable Labeling for Cytoarchitectonic Characterization of Large Optically Cleared Human Neocortex Samples (springer.com)