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Whole Mouse Brain Delipidation - LifeCanvas Active

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

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Abstract

This LifeCanvas SmartBatch+ protocol is intended for whole mouse brain tissue clearing and incorporates electrophoretic delipidation. While this delipidation protocol is longer than other delipidation protocols we tested, it preserves the endogenous fluorescence of the tissue and clears the whole brain most effectively, demonstrating the morphology of fine cell structures deep in the brain.

It is important to note that while most of our protocol is derived from the original LifeCanvas SmartBatch+ protocol, our protocol has some modifications. Most notably, the SmartBatch+ settings (temperature, time, voltage). While these settings have been found to reliably delipidate whole brain samples, the concern of precipitant build-up and damage to the clearing cup should be acknowledged. Therefore, it is imperative to conduct spill tests on the clearing cup, effectively drain out the conduction buffer, and clean the SmartBatch+ equipment with distilled water after every run.

This delipidation protocol should be followed with a refractive index matching protocol (see: **Refractive Index Matching - EasyIndex**) and whole brain imaging (see: **SmartSPIM setup and alignment** and **Imaging cleared mouse brains on SmartSPIM**).

Materials

Reagents:

LifeCanvas SHIELD Reagent Kit **Catalog #**SH-250

- SHIELD ON **Catalog #**(SH-ON)
- SHIELD Epoxy **Catalog #**(SH-Ex)
- SHIELD Buffer **Catalog #**(SH-BS)

Nuclease-free water (e.g. MilliQ or HPLC grade water)

Conduction Buffer **Catalog #**CB

Delipidation Buffer **Catalog #**DB

Optional reagents:

10xPBS **Ambion Catalog #**AM9624

Sodium Azide 5% **Ricca Chemical Company Catalog #**71448-16

Materials:

	Materials	Product number
	Kimwipes	Millipore Sigma, Z188956-60PAK
	Aluminum foil	Amazon, B074NB5CDZ
	50ml falcon conical tube	Fisher Scientific, 14-432-22
	Shaker	OHAUS, 404P77
	Metal spatula	Cole Parmer, ux-06287-07
	50mL serological pipettes	ThermoFisher, 170376N
	Serological pipette filler	ThermoFisher, 9501
	Stir plate	MilliporeSigma, Z693510
	Magnetic stirring bar	MilliporeSigma, Z328839
	Pyrex round media storage bottle	MilliporeSigma, CLS13951L

	Materials	Product number
	SmartBatch+ accessory kit (includes incubation jar, ring stand, sample ring, mesh bags, lock ring	LifeCanvas, SB-AK

Equipment

SmartBatch+ — NAME

Electrophoretic tissue clearing & immunolabeling chamber — TYPE

LifeCanvas Technologies — BRAND

SB — SKU

https://lifecanvastech.com/products/smartbatch/ — LINK

Stochastic electrotransport (SE) — SPECIFICATIONS

Equipment

Clearing Cup

NAME

Clearing Cup for Smartbatch+

TYPE

LifeCanvas Technologies

BRAND

CC

SKU

https://lifecanvastech.com/products/smartbatch/

LINK

SmartBatch+ Accessories

SPECIFICATIONS

PDF

Equipment

Mesh Bags

NAME

Mesh Bags to hold whole mouse brains

TYPE

LifeCanvas Technologies

BRAND

MB

SKU

https://lifecanvastech.com/products/smartbatch/

LINK

SmartBatch+ Accessories

SPECIFICATIONS

PNG

Equipment

Heratherm General Protocol Microbiological Incubator	NAME
Incubator	TYPE
ThermoFisher	BRAND
51028063	SKU
https://www.thermofisher.com/order/catalog/product/51028130	LINK
75 L	SPECIFICATIONS

Equipment

MaxQ™ HP Incubated Tabletop Orbital Shaker	NAME
MaxQ™ HP, 120 V 60 Hz, 6,5 A o 230 V 50/60 Hz, 3,2 A	TYPE
Thermo Scientific	BRAND
SHKE420HP	SKU
https://www.thermofisher.com/order/catalog/product/SHKE420HP	LINK

Recipes:

LifeCanvas SHIELD OFF Solution

Combine the following reagents in the order listed below and stir for 10 minutes. Mix solution over ice or move solution to 4c storage after mixing.

20mL of LifeCanvas SHIELD OFF Solution is required for processing one brain.

	A	B
	Nuclease-free water (e.g. MilliQ or HPLC grade water)	5mL
	LifeCanvas SHIELD Buffer	5mL
	LifeCanvas SHIELD Epoxy	10mL
	Total	**20mL**

Optional recipes:

1L 1xPBS:

Combine the following reagents into a container with a stir bar. A graduated cylinder may be used to measure MilliQ water and a graduated cylinder or serological pipet may be used to measure 10xPBS. Mix well on a stir plate at high speed (300 RPM or higher) for 00:02:00 or until solution is mixed. Store at Room temperature for 1 month.

	Reagent	Volume
	Milli-Q water	900mL
	10xPBS	100mL

1L 1xPBS & 0.02% Sodium Azide:

Combine the following reagents into a container with a stir bar, using a graduated cylinder to measure the 1xPBS and a P5000 pipette to measure the 5% sodium azide. Mix well on a stir plate at high speed (300 RPM or higher) for 00:02:00 or until solution is mixed. Store at Room temperature or 4 °C for up to 1 year.

	Reagent	Volume
	1xPBS	996mL
	0.02% Sodium Azide	4mL

Safety warnings

Make sure to complete all steps with appropriate PPE: gloves, eye protection, lab coat. While it is not known what is in the LifeCanvas reagents for proprietary reasons it is still vital to use PPE when handling all reagents.

Sodium Azide is toxic and carcinogenic. It should be handled and prepared with care. Do not breathe dust, do not use metal utensils. Wear gloves when handling this chemical.

All reagents must be placed in their respective waste bottles and disposed of properly. Please contact Environmental Health & Safety if you need safety labels or more information on properly disposing of all of the LifeCanvas reagents.

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

The specimen used for this protocol is a perfused mouse brain fixed in 4% paraformaldehyde. See **Mouse Cardiac Perfusion Fixation and Brain Collection V.5** for perfusion and fixation instructions.

Brains should immediately be either stored or index matched when unloaded from SmartBatch+ after delipidation in step 5 of this protocol. If proceeding to index matching, it will be helpful to prepare the 50% EasyIndex solution ahead of time from the protocol **Refractive Index Matching - EasyIndex**.

Tissue Preservation with SHIELD OFF & SHIELD ON

4d 0h 15m

1 **SHIELD OFF Preservation:**

Safety information

Make sure to complete all steps with gloves on, while it is not known what is in these reagents due to proprietary reasons it is still vital to use gloves when handling all reagents.

1.1 Prepare 20 mL of fresh SHIELD OFF solution:

Note

The following instructions provide a total volume of 20 mL, which is sufficient to process a single brain specimen. Brains may be processed in batches of 12 or less. If processing more than one brain, 5 mL of nuclease-free water, 5 mL of SHIELD buffer solution, and 10 mL of SHIELD epoxy per brain will be needed, and the amounts of these reagents should be recalculated.

	Reagent	Amount needed
	Nuclease-free water (e.g. MilliQ or HPLC grade water)	5mL
	LifeCanvas SHIELD buffer	5mL
	LifeCanvas SHIELD epoxy	10mL
	Total	**20mL**

1.2 Place a pyrex storage bottle or similar glass container for mixing the total amount of SHIELD OFF solution needed into a bed of wet ice. Measure the correct amounts of the following reagents in this order into the glass container: nuclease-free water (e.g. MilliQ or HPLC grade water), SHIELD buffer, and SHIELD epoxy. Mix on stir plate for 00:10:00 .

10m

1.3 Place each brain into a 50 mL conical tube, add 20 mL of SHIELD OFF, and cover the tube with aluminum foil to protect the tissue from light. Conical tubes should be kept upright in a tube rack throughout this protocol.

> Note
>
> The whole mouse brain in the 50 mL conical tube will be completely submerged in the SHIELD OFF solution.

1.4 Place 50 mL conical tube in 4 °C fridge with the shaker at a low shaking speed. 3d
Leave the conical tube containing the brain and SHIELD OFF in 4 °C fridge with the shaker for 72:00:00

2 **SHIELD ON Preservation:**

2.1 Pre-Warm SHIELD ON Buffer by placing in 37 °C oven for 00:05:00 . 5m

2.2 Remove the 50 mL conical tubes containing the brain specimen in SHIELD OFF from the 4 °C fridge. Remove SHIELD OFF solution from the conical tube by scooping brain out of tube with a spatula and pouring SHIELD OFF solution into appropriate waste receptacle. Replace the brain in the tube and add 20 mL of SHIELD ON solution into the tube using a serological pipet.

> Note
>
> While pipeting reagents into tube containing the brain, point tip of pipet at the inner side wall of tube so that the initial force of the reagent stream hits the tube wall and not the brain.

2.3 Place 50 mL conical tubes with 20 mL of SHIELD ON solution in oven at 37 °C with shaking for 24:00:00 . Tubes should be upright inside a tube holder. 1d

2.4 SHIELD Preservation is now complete. At this point, the sample can either proceed to Passive Delipidation (step 3) or be stored in 20 mL 1X PBS with 0.02% sodium azide at 4 °C for several months without significant loss of fluorescence signal and structural integrity. To store in 1XPBS with 0.02% sodium azide, remove the SHIELD ON solution from the conical tube by scooping brain out of tube with a spatula and pouring SHIELD ON solution into appropriate waste receptacle. Replace the brain in the tube and add 20 mL of 0.02% sodium azide into the tube using a serological pipet.

Safety information

Sodium Azide is toxic and carcinogenic. It should be handled and prepared with care. Do not breathe dust, do not use metal utensils. Wear gloves when handling this chemical.

Passive Delipidation

1d 18h

3 **Passive Delipidation:** 1d

Incubate the sample in Delipidation Buffer for 24:00:00 at 45 °C on a shaker at slow shaking speed. If you do not have a 45 °C incubator, 37 °C will suffice. The step can be extended three extra nights if needed (example: over the weekend), but do not skip it.

If processing a single sample, you may complete the passive delipidation step in the 50mL conical tube. See step 3.1.

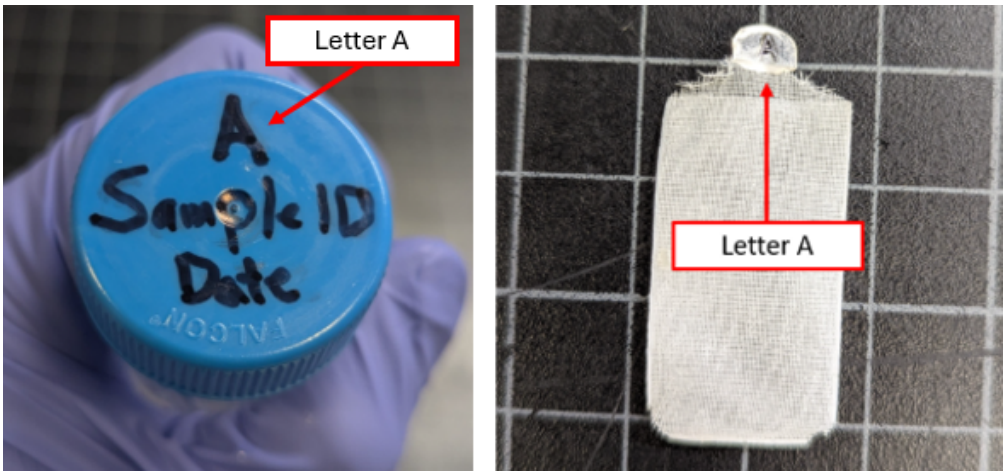
If processing multiple samples together as a batch, complete the passive delipidation step using the incubation jar. See step 3.2.

3.1 Remove SHIELD ON solution from the conical tube (or 1XPBS & 0.02% sodium azide solution, if the sample was in storage) by scooping brain out of tube with a spatula and pouring SHIELD ON solution into appropriate waste receptacle. Replace the brain in the tube and add 20 mL of Delipidation Buffer into the tube using a serological pipet. The sample is now ready for incubation.

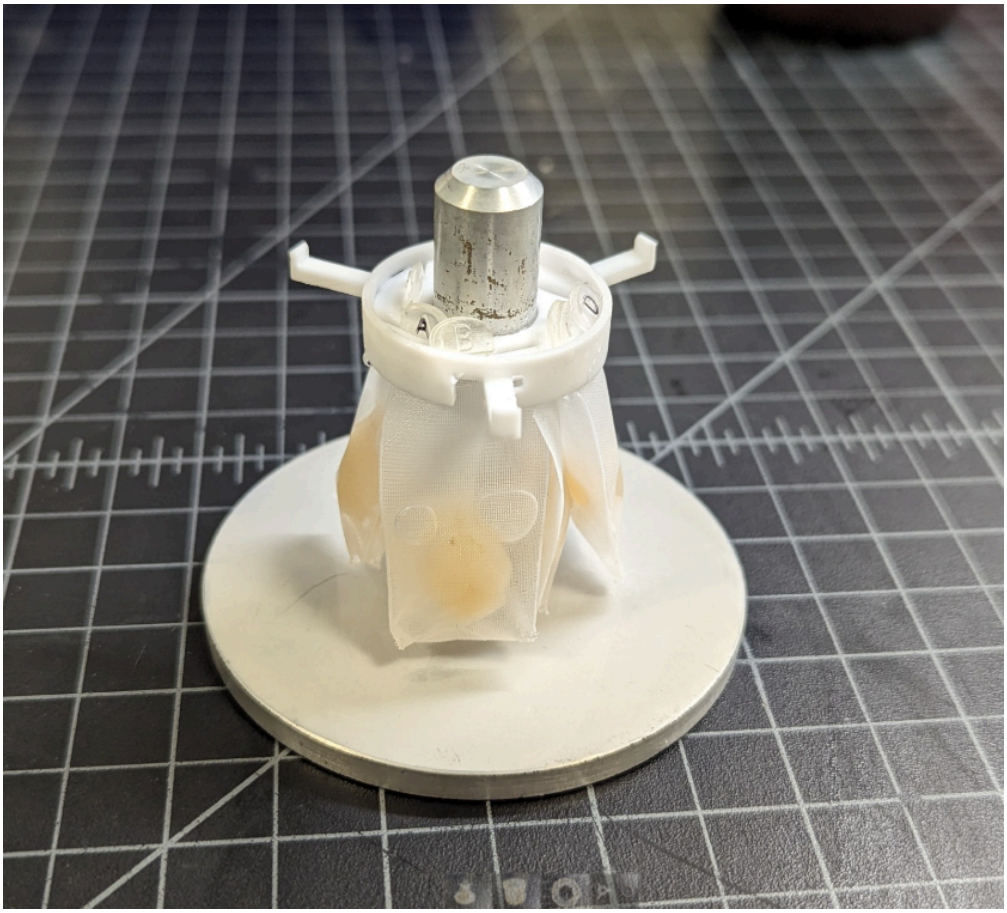
3.2 Remove SHIELD ON solution from the conical tube (or 1XPBS & 0.02% sodium azide solution, if the sample was in storage) by scooping brain out of tube with a spatula and pouring SHIELD ON solution into appropriate waste receptacle. Place each mouse brain

sample into a lettered mesh bag and hang on metal Ring Stand. When all samples are loaded onto the Ring Stand, lock them in place with the plastic Lock Ring. To track the sample that is in the incubation jar and prevent mix-up between samples, write down the sample ID with the corresponding letter of the mesh bag.

Place the Ring Stand with the samples in the mesh bags into the Incubation Jar. Fill the Incubation Jar with 250 mL of Delipidation Buffer. The samples are now ready for incubation



Example: Mesh bag labeled with the letter "A" (pictured right), corresponds to "A" written on the lid of the conical tube containing the brain specimen, along with the sample ID and date.



Mouse brain samples in lettered mesh bags on metal Ring Stand

Note

Replace Delipidation Buffer in Incubation Jar after every 10 batches.

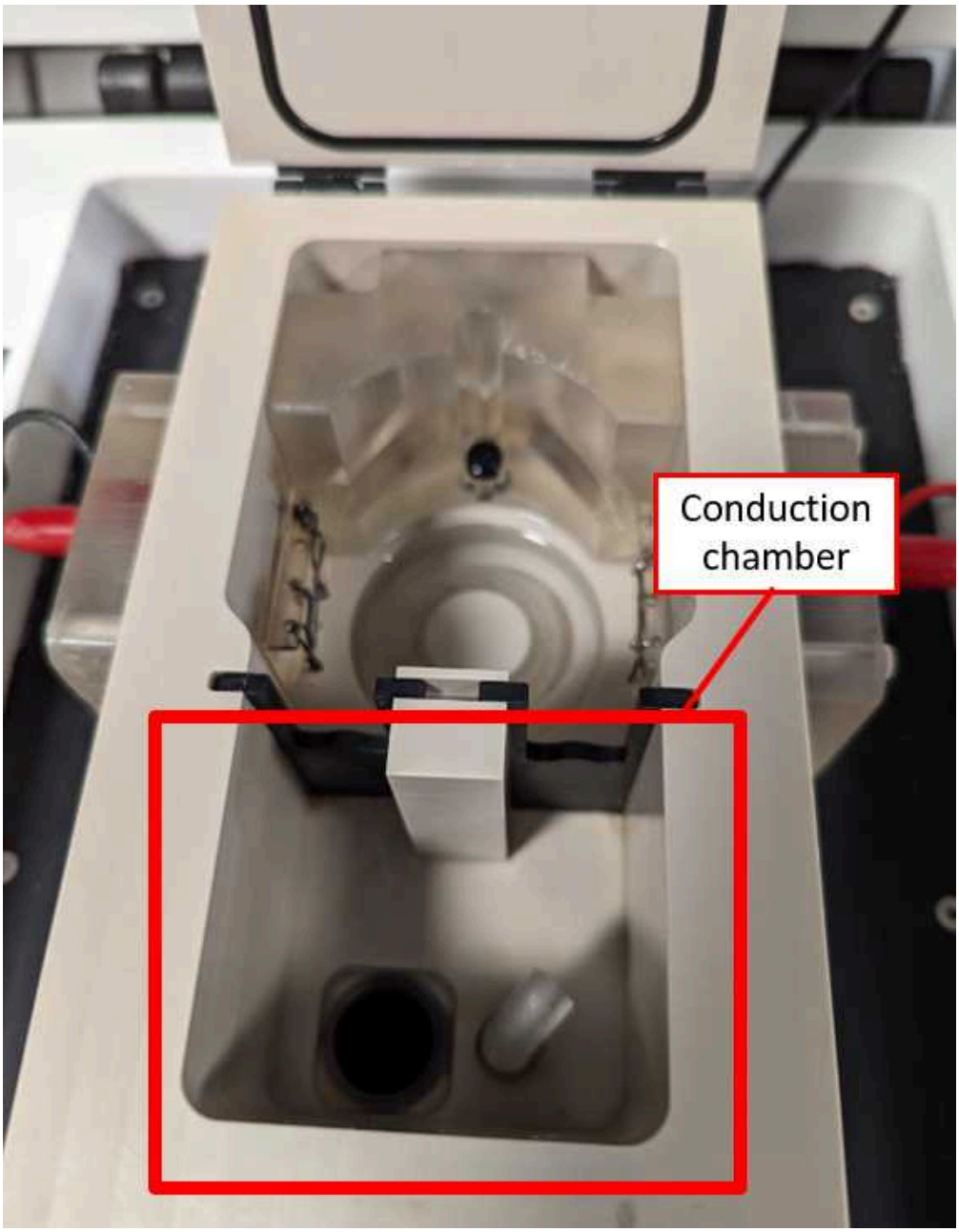
Active Delipidation

3d 12h

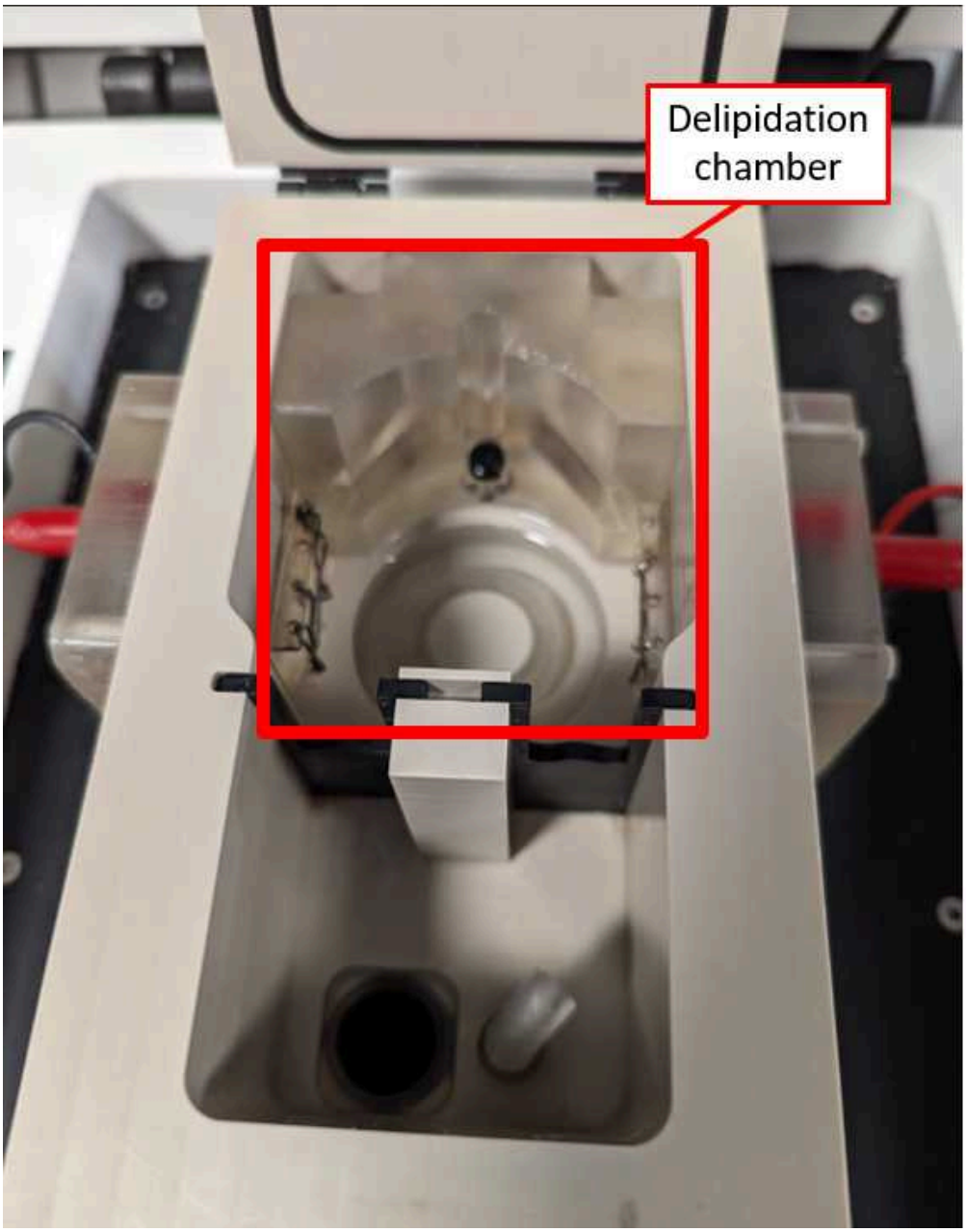
4 **Electrophoretic Delipidation using SmartBatch+ and SmartBatch+ Set Up:**

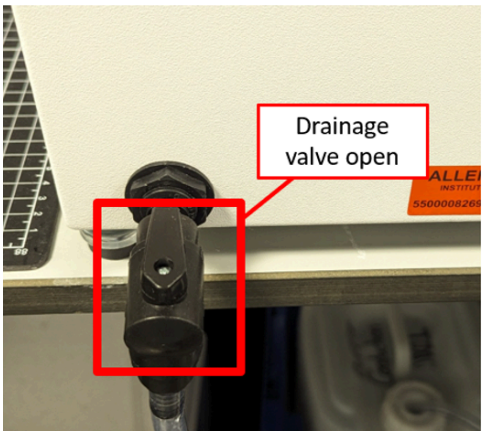
4.1 Make sure Auxiliary Power on SmartBatch+ device control panel is turned off.

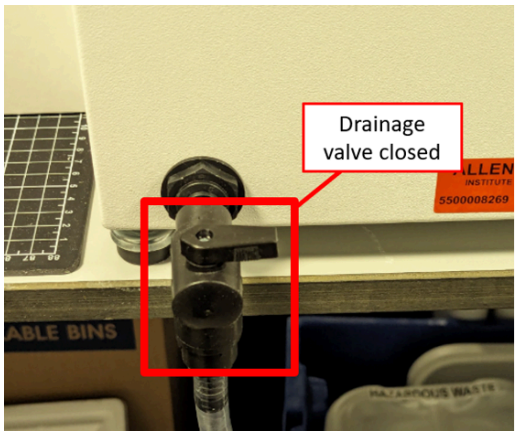
4.2 Before using, wash SmartBatch+ Conduction Chamber by pouring 450 mL Milli-Q water into Chamber and draining through open drainage valve. Repeat 3 times.



4.3 Drain out any liquid from the device and ensure the Drainage Valve is closed. Soak up any remaining delipidation buffer from the Delipidation Chamber using a paper towel and dispose of paper towel in appropriate waste.


Delipidation
chamber





4.4 Pour a whole 450 mL bottle of Conduction Buffer into the Conduction Chamber. (see figure in step 4.2)

Note

Conduction Buffer may be re-used for up to 168 hours of electrophoretic delipidation in SmartBatch+. Retain bottle for storage of recycled Conduction Buffer. (see step 7.1)

4.5 Remove the Clearing Cup from its storage container and wash carefully with a gentle stream of Nuclease-free water.

Note

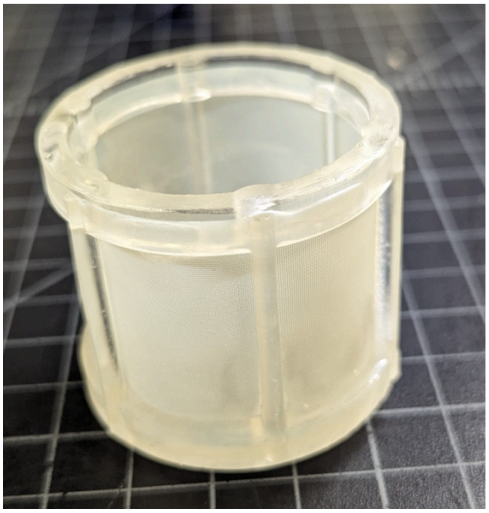
It is important to keep the membrane of the Clearing Cup hydrated at all times.

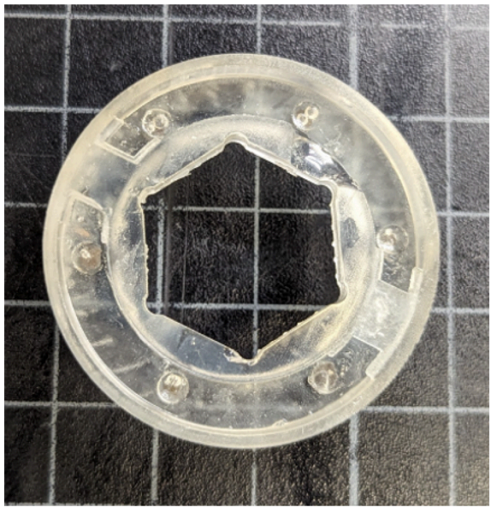
Clearing Cup is fragile, handle gently for all steps. Do not touch the mesh sides of the Clearing Cup. Instead, hold the cup by the solid plastic top rim or plastic bottom.

4.6 Place the Clearing Cup into a beaker of Nuclease-free water for several minutes to finish washing the cup.

4.7 Remove the Clearing Cup from the beaker and dump out any water. Carefully use a kimwipe to soak up any remaining water inside.

4.8 Insert the pegs on the bottom of the Clearing Cup into the grooves on the Chamber Hex Piece. Twist the Chamber Hex Piece to lock these two pieces together.





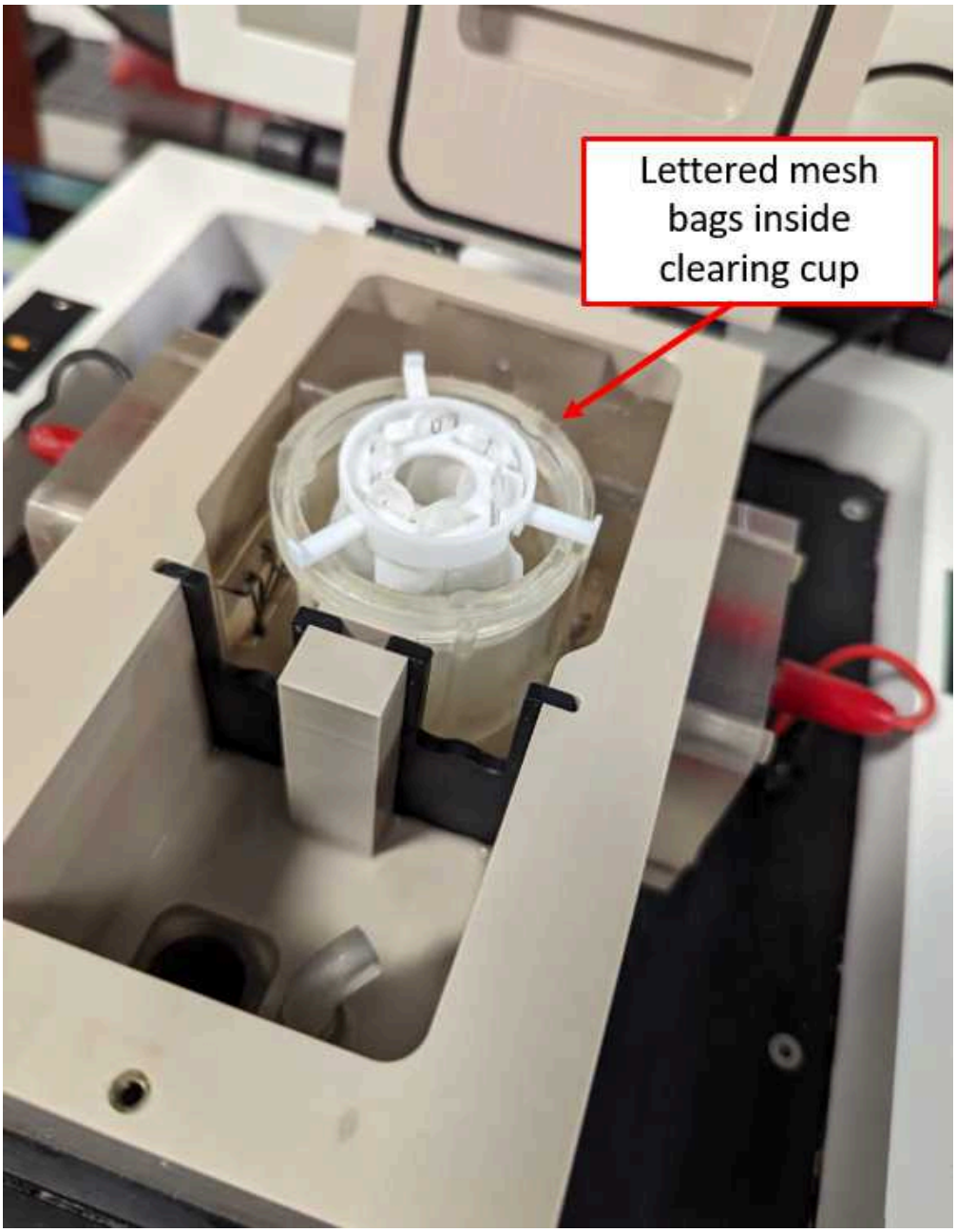
Place Clearing Cup (pictured left) on top of Chamber Hex Piece (pictured right).

4.9 Place the Clearing Cup into the Delipidation Chamber. (see figure in step 4.3)

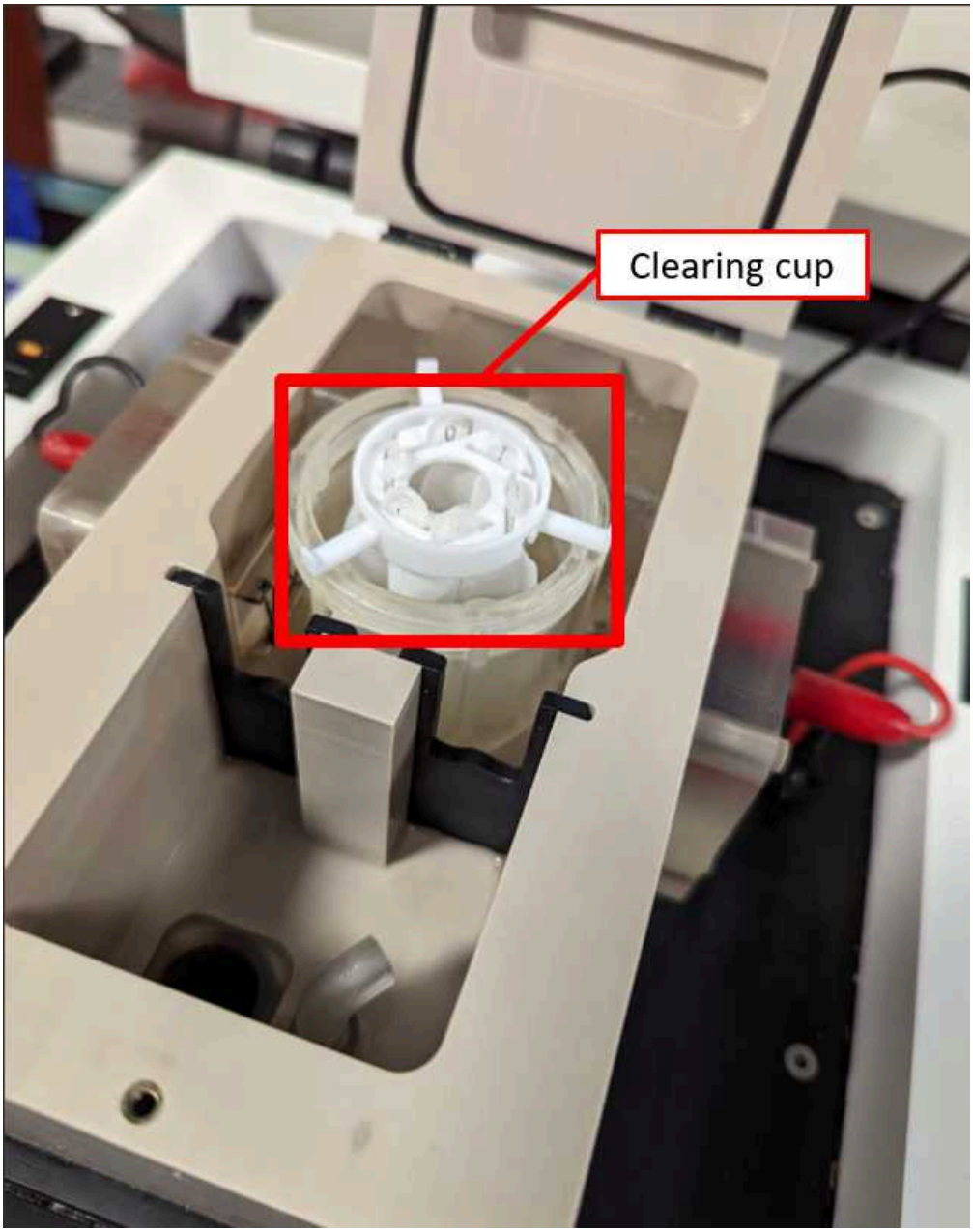
4.10 Remove the Sample Ring with the lettered mesh bags containing the brain specimens from the Ring Stand in the Delipidation Jar (from step 3) and place it inside the Clearing Cup. All the mesh bags should fit inside the cup and the ends of the three plastic arms protruding from the sample ring should rest on the edges of the cup.

Note

If sample was processed individually in a 50 mL conical tube for Passive Delipidation (step 3), first transfer the sample into a lettered mesh bag and lock the bag into place on the Sample Ring using the Ring lock (see step 3.2 for details), then proceed to step 4.10.

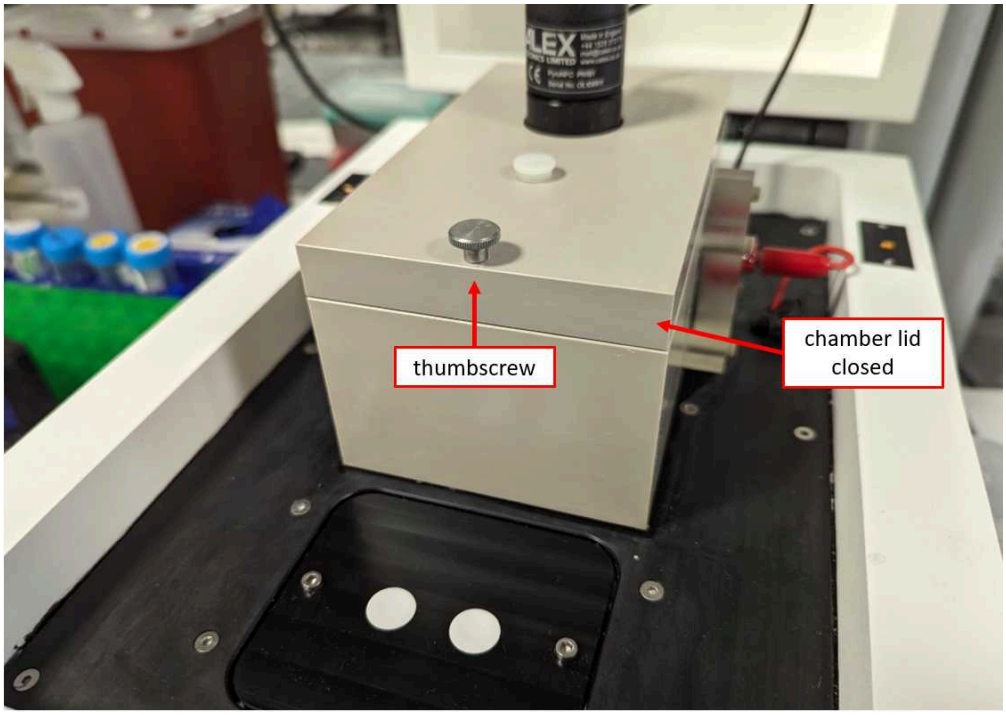


4.11 Fill the Clearing Cup with 35 mL of Delipidation Buffer. All brain samples should be completely submerged in Delipidation Buffer.



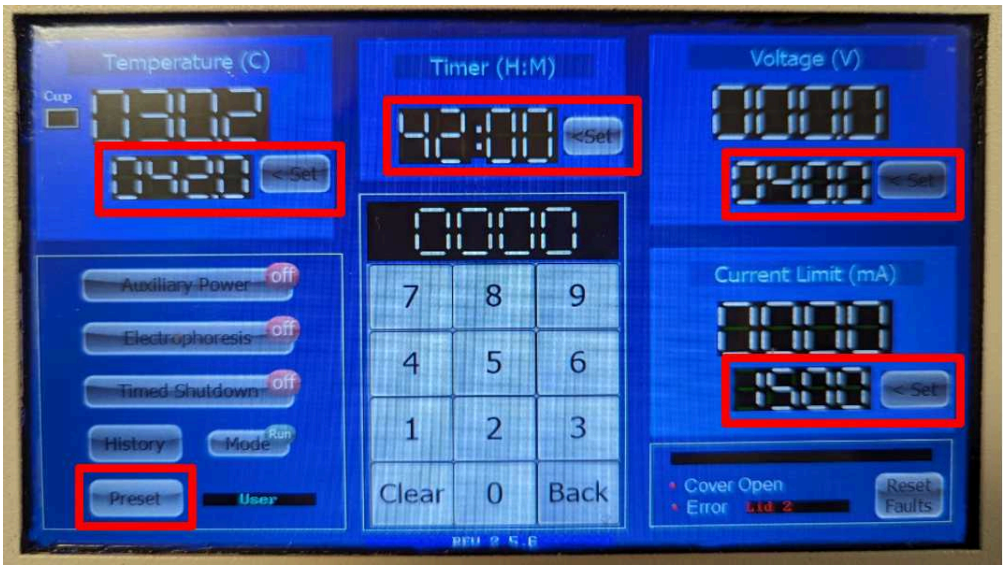
4.12 Turn on Auxiliary Power on control panel.

4.13 Close the Chamber Lid and secure it with the thumbscrew.

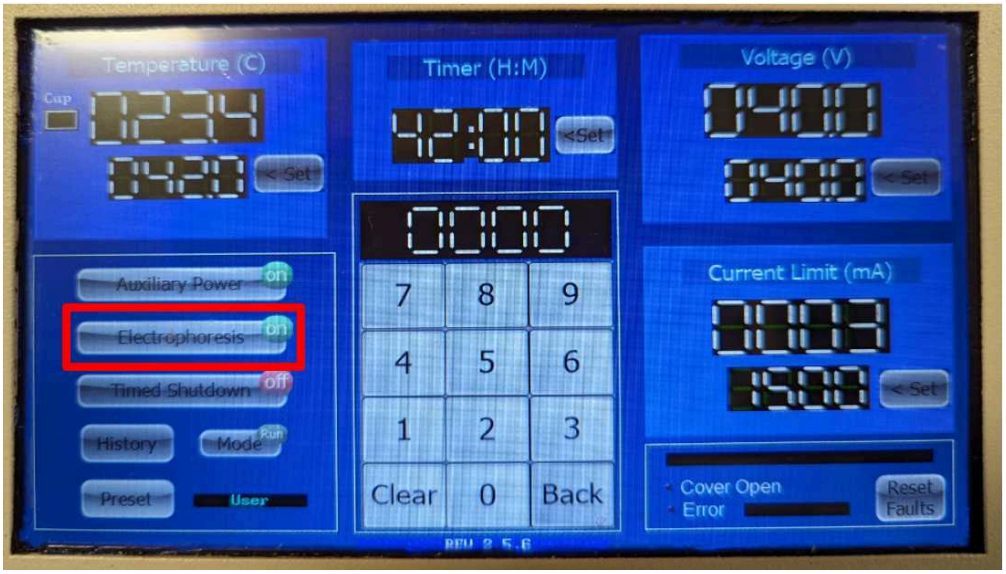


4.14 Close the Case Lid.

4.15 Press “Preset” until it indicates Clearing Mode. Change settings to **40V, 1500 mA current limit,** 42:00:00 **, and** 42 °C **.** 1d 18h



4.16 Turn on the Electrophoresis Power on control panel.



4.17 Mark the time when this active delipidation step started or set a timed shutdown for 42:00:00 on control panel. 1d 18h

5 Unloading Brain Specimens from SmartBatch+:

Note

Brains should immediately be either stored or index matched when unloaded from SmartBatch+ after delipidation. If proceeding to index matching, it will be helpful to prepare the 50% EasyIndex solution ahead of time from the protocol **Refractive Index Matching - EasyIndex**.

5.1 After electrophoretic delipidation finishes running, open the Case Lid and Chamber Lid. Lift the Sample Ring out of the Clearing Cup and place it on the Ring Stand.

5.2 One at a time, remove each brain from its mesh bag hanging on the Ring Stand. Note the letter on the mesh bag and place the brain inside a 50mL conical tube. Label the tube with the corresponding brain specimen ID (see step 3.2).

Immediately store brains (proceed to step 5.3) or immediately begin index matching. Refer to **Refractive Index Matching - EasyIndex** protocol for instructions on index matching.

5.3 To store brains after delipidation, fill each 50 mL tube with 20 mL 1xPBS + Azide 0.02% and place in 4 °C . Brains may be stored for 2-3 months.

SmartBatch+ Clean Up

1w 4d 4h 5m

6 **Unloading Clearing Cup from SmartBatch+:**

6.1 Remove the Clearing Cup from the Delipidation Chamber and empty the Delipidation Buffer from Clearing Cup into appropriate waste receptacle. Unscrew the Chamber Hex Piece from the Clearing Cup.

6.2 Test the Clearing Cup for leakage: 5m

Gently pat the solid plastic base of Clearing Cup dry with a kimwipe and carefully fill with Nuclease-free water. Place Clearing Cup on a paper towel and leave for 00:05:00 .

If the paper towel is dry and there is no evidence of leakage, empty Clearing Cup and replace in storage solution.

If the paper towel is wet, this may indicate Clearing Cup leakage. Repeat this step, and if the paper towel is consistently wet, use a new Clearing Cup next time using SmartBatch+.

7 **Drain Conduction Buffer from SmartBatch+:**

7.1 Conduction Buffer may be re-used for up to 168:00:00 running in SmartBatch+ electrophoretic delipidation. Drain Conduction Buffer into bottle to retain for extra use if Buffer has been used in SmartBatch+ less than 100:00:00 . 1w 4d 4h

Protocol references

LifeCanvas SmartBatch+ User Manual