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iDISCO protocol for whole-mount immunostaining and volume imaging

Clara Huesing[1], Heike Muenzberg[1], David Burk[1], Hayden Torres[1]

[1]Pennington Biomedical Research Center

1 Works for me dx.doi.org/10.17504/protocols.io.wzuff6w

SPARC
Tech. support email: info@neuinfo.org

Clara Huesing
Pennington Biomedical Research Center

ABSTRACT

Protocol documents how to dehydrate, immunolabel, and clear tissue in preparation for light sheet microscopy.

EXTERNAL LINK

https://idisco.info/idisco-protocol/update-history/

DOI

dx.doi.org/10.17504/protocols.io.wzuff6w

EXTERNAL LINK

https://idisco.info/idisco-protocol/update-history/

PROTOCOL CITATION

Clara Huesing, Heike Muenzberg, David Burk, Hayden Torres 2021. iDISCO protocol for whole-mount immunostaining and volume imaging. **protocols.io** https://dx.doi.org/10.17504/protocols.io.wzuff6w

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

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PROTOCOL INTEGER ID

19220

MATERIALS TEXT

MATERIALS

Normal Donkey Serum **Contributed by users** **Catalog #017-000-121**

Triton X-100

Sigma Catalog #93426

10xPBS **Ambion Catalog #AM9624**

Tween-20 **Sigma**

Aldrich Catalog #P9416

DMSO **Fisher**

Scientific Catalog #67-68-5

Sodium azide **Sigma**

Aldrich Catalog #S2002

Glycine **Sigma**

Aldrich Catalog #G7126-500g

Heparin sodium salt from porcine intestinal mucosa **Sigma**

Aldrich Catalog #h3393-50KU

Methanol **Fisher**

Scientific Catalog #A412SK-4

Dichloromethane **Sigma**

Aldrich Catalog #270997-12x100ml

Benzyl ether (Dibenzyl ether) **Sigma**

Aldrich Catalog #108014-1KG

Paraformaldehyde 16% **Electron Microscopy**

Sciences Catalog #15710

BUFFERS:

PTx.2 (1L)

- 100mL PBS 10x
- 2mL TritonX-100
- Volume to 1L with dH2O

PTwH (1L)

- 100mL PBS 10X
- 2mL Tween- 20
- 1mL of 10mg/mL Heparin stock solution
- Volume to 1L with dH2O

Permeabilization Solution (500mL)

- 1X PBS = 300mL
- 0.2% Triton X-100 = 100mL
- 11.5 g Glycine = 11.5g
- 20% DMSO = 100mL

Blocking Solution (500mL)

- 420mL PTx.2
- 30mL Donkey Serum
- 50mL DMSO

Primary Incubation Solution (500mL)

- 25mL DMSO
- 15mL Donkey Serum
- 460mL PTwH

Secondary Incubation Solution (500mL)

- 15mL Donkey Serum
- 485mL PTwH

Consumables and hardware

Tubes Eppendorf 5mL

Orbital shaker VWR nutating mixer Hybridization oven: VWR 5420

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With carousel: 47746-112
50ml Chemical Resistant Plastic Tubes- Sarstedt 62.547.004
Millex .22µm syringe driven filter unit- Millipore SLGP033RS
60ml Luer-Lok Syringe- Fisher 309653

SAFETY WARNINGS

All handling of dichloromethane (DCM), methanol (MeOH), and dibenzyl ether (DBE) should be done under a chemical hood to avoid vapor inhalation. All three chemicals are organic solvents, so appropriate precautions should be taken, such as wearing a lab coat and utilizing chemical resistant gloves and funnels to prevent splashing.
DCM dissolves plastic! This means you will need to use glassware when working with DCM (i.e. glass graduated cylinders, beakers, pipettes, jars, funnels)
The DCM/MeOH mixture used in the methanol pretreatment and clearing steps is a volatile solution. As you unscrew the lids to jars containing this mixture, cover the lid with kimwipes to prevent chemical spray.

Environment friendly tip:The volume of solution one uses in any step of this protocol should be calculated using the **minimum** amount of liquid it takes to fully submerge your sample in order to reduce organic waste.

BEFORE STARTING

Read safety warnings before starting.

Methanol Pretreatment

1 a. Clean workspace
b. Dehydrate samples with a series of MeOH/H2O washes:
20% MeOH/ 80% H2O
40% MeOH/ 60% H2O
60% MeOH/ 40% H2O
80% MeOH/ 20% H2O
100% MeOH
100% MeOH
01:00:00 each wash. Place samples on rocker at room temperature during washes.

***Before continuing to step 1c, transfer samples into glass vials (DCM safe)**
b. Incubate overnight with agitation (rocker) in 66% DCM/33% MeOH at room temperature

2 a. Prepare plastic tubes and fill with 100% methanol
b. Once the DCM/MeOH incubation is complete, transfer samples into plastic tubes made in 1a.
c. Wash 2x in 100% methanol (**00:15:00** for each wash) at room temperature. Place on rocker during washes
d. Transfer samples to a 1:5 concentration solution of 30% H2O2:100% MeOH and incubate in **4 °C** fridge overnight

3 a. Pour out H2O2/MeOH mixture and rehydrate samples with a series of Methanol/H2O washes
80% MeOH/ 20% H2O
60% MeOH/ 40% H2O
40% MeOH/ 60% H2O
20% MeOH/ 80% H2O
100% PBS
01:00:00 each wash. Place samples on rocker at room temperature during washes.
b. Incubate samples in fresh PBS overnight on rocker, at room temperature

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4 a. Pour out PBS and wash samples 2x in PTx.2 buffer, 1hr each wash on rocker, at room temperature
b. Continue to immunolabeling (step 5).

Immunolabeling

5 a. Incubate samples at **37 °C** in permeabilization solution on rocker, for 2 days

6 Pour out permeabilization solution and incubate samples at **37 °C** in blocking solution on rocker, for 2 days

7 a. Mix the primary antibody and primary incubation solution

Tip: Refrigerate the solution at **4 °C** while you are doing step 7b.

b. Pour out blocking solution that your samples are submerged in
c. Transfer your samples to fresh tubes and submerge them in solution you created in 7a.
d. Incubate for 1.5 - 2 weeks on rocker, at room temperature

Tip: turn tubes on their side to ensure that the samples are getting adequately “washed” with the solution

8 a. Pour out the primary antibody + primary incubation solution mixture
b. Wash 5x in PTwH, **01:00:00** each wash on rocker, at room temperature
c. Incubate in fresh PTwH overnight on rocker, at room temperature

9 a. Mix the secondary antibody and secondary incubation solution
b. Filter the solution you made in 9a with a 50mL syringe that has a 0.22um filter attached

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Figure 1 - transferring solution from beaker in right corner to clean beaker in middle of pic.

Note: Filtering the secondary antibody solution reduces aggregation of antibodies that results in autofluorescent puncta that appears within the tissue during imaging

c. Pour out PTwH, transfer samples to fresh tubes, and pour your filtered solution over them
d. Cover the samples in aluminum foil to protect from photobleaching
e. Incubate samples in solution for 2 weeks on rocker, at room temperature
Tip: Turn tubes on their side to ensure that the samples are adequately getting “washed” with the solution

10 a. Pour out secondary antibody + secondary incubation solution mixture
b. Wash samples 5x in PTwH, **01:00:00** each wash on rocker, at room temperature
c. Incubate samples in fresh PTwH overnight on rocker, at room temperature

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Clearing

11 a. Dehydrate samples with a series of MeOH/H2O washes:
20% MeOH/ 80% H2O
40% MeOH/ 60% H2O
60% MeOH/ 40% H2O
80% MeOH/ 20% H2O
100% MeOH
100% MeOH
01:00:00 each wash. Place samples on rocker at room temperature during washes.
b. Incubate samples in 100% MeOH overnight on rocker, at room temperature

12 a. Make your 66% DCM/33% MeOH mixture and pour into **glass jars**

Note: the following steps describe how to "sandwich" your samples. We have found this makes larger specimens more manageable for light sheet microscopy. For example, to prevent excessive curling of the spine in our thoracic samples, we use this technique to straighten out the sample. This reduces the plane depth of the specimen, which helps with imaging larger specimens.

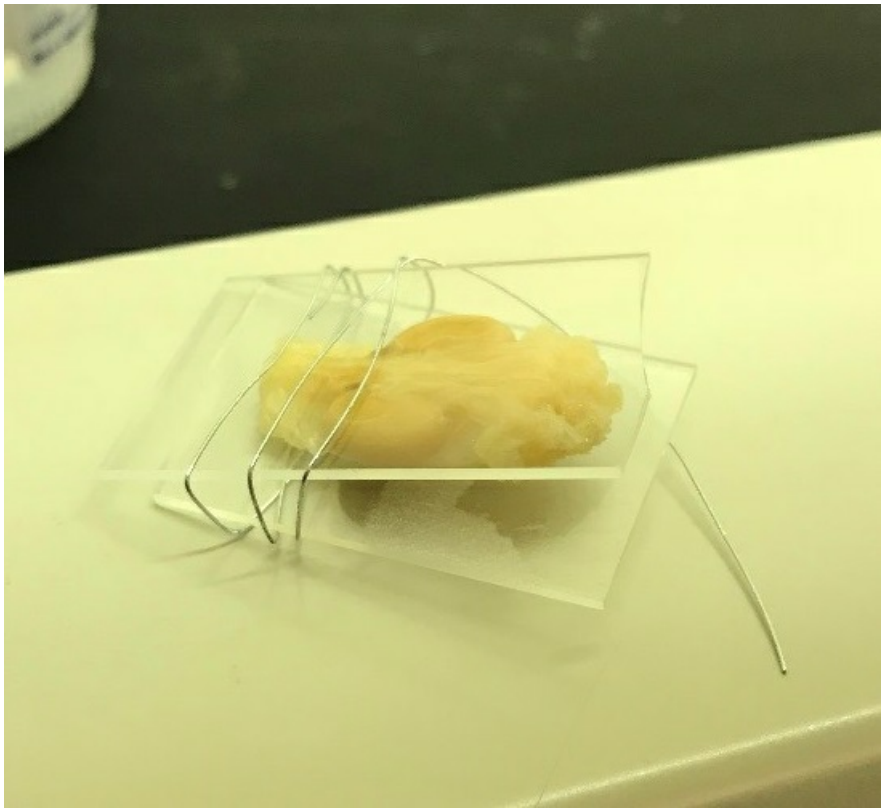

Figure 2

b. Press samples between glass slides and secure in place by wrapping wire around the sandwich (Figure 2). Use enough force to secure the sample, but do not flatten sample to a point where you are altering the physiological shape! Do not use this method on soft tissue.
c. As you complete a sample, place it into its corresponding glass jar that has the 66% DCM/33% MeOH mixture, place on rocker
d. Incubate samples in the 66% DCM/33% MeOH mixture for **03:00:00** on rocker, at room temperature

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e. While samples are incubating, fill fresh glass jars with 100% DCM
f. Once incubation is complete, unwrap the wire used to hold the sandwich together using two pairs of forceps – this minimizes contact with DCM as you do not want to touch DCM with only bare hands or nitrile gloves!
g. As you free up a sample, place it into its corresponding jar that you have filled with 100% DCM
h. Wash samples 2x in 100% DCM, **00:15:00** each wash, on a rocker at room temperature
i. While samples are washing, fill fresh glass jars to the top with dibenzyl ether (DBE) – this is what your sample will be suspended in for long-term storage
j. After step 2h. is complete, transfer samples to their corresponding glass jars that you have filled with DBE

Note: Transferring samples from 100% DCM to DBE is time sensitive. The sample will begin to dry out very quickly once removed from 100% DCM.

k. Thoroughly clean workspace

Following dehydration, samples can be stored in 100% MeOH for a short period of time (roughly two days). When hydrated, samples can be stored in PBS w/azide. Clearing can also be reversed if needed.

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