HCBI CLARITY PROTOCOL

Solutions:

Hydrogel Solution

3% Paraformaldehyde
3% (w/v) Acrylamide
0.02% (w/v) Bisacrylamide
2.5 mg/mL VA044 Thermal Polymerization Initiator

Made in PBS

PROTOS clearing solution (from the Chung lab website – MIT)

23.5% (w/v) n-methyl-d-glucamine
29.4% (w/v) diatrizoic acid
32.4% (w/v) iodixanol (same as Histodenz, Omnipaque)

Made in H2O

Optiview clearing solution (http://hcbi.fas.harvard.edu/publications/optimized-protocol-imaging-cleared-neural-tissuesusing-light-microscopy)

172 mM sodium diatrizoate
815 mM meglumine diatrizoate
0.5 mM EDTA
0.1 % Tween-20, pH 8

Made in H2O

(refractive index adjusted to 1.45 using a refractometer, filtered through 0.22 µm pore filter)

Do not use heat when mixing the solution, as this will cause a color change. This solution should be stored carefully to ensure that no water is lost, as just a small amount of evaporation will result in precipitation and RI change. Teflon tape can be used to increase the security of the bottle's seal, and parafilm can be used around the cap.

Protocol

1) **Perfusion:**
    a) Perfuse the animal with 25-50mL of PBS
    b) Perfuse the animal with 25mL of Hydrogel Solution (optional)
    c) Dissect out tissue of interest

d) Submerge tissue in 10-20 mL of Hydrogel Solution for 24hrs at 4C.

2) **Hydrogel polymerization**
   a) Place tissue in X-CLARITY hydrogel polymerization device using the following settings:
      i) Vacuum: -90 kPa
      ii) Temperature: 37C
      iii) Timer: 03:00 - 04:00 hrs
      iv) Vessel type: Tube (for large tissues in 50mL conicals, Well Plate (for tissue slices in multi-well plates).
   b) Submerge sample in PBS overnight

3) Passive pre-clearing (optional)
   a) Submerge sample in 10-20mL of ETC buffer (Logos Biosystems) overnight

4) Electrophoretic lipid extraction
   a) Place sample in X-CLARITY ETC chamber.
   b) Fill reservoir with 1.2 L of ETC solution.
   c) Run system at 1.0 A and 37 C for:
      i) 8 hours (1-2 mm tissue slice)
      ii) 24 hours (small organ – i.e. mouse kidney or half brain)
      iii) 24-48 hours (large organ – i.e. whole mouse brain) ** Exchange ETC solution after 24 hrs!!
   d) Incubate sample in 10-20 mL of PBST overnight

5) Immunostaining (optional)
   a) Immunostaining is not recommended on tissues greater than 2 mm in their thinnest dimension
   b) Follow the immunolabelling protocols from the iDISCO plus paper (Renier et al., Cell, 2016)
   c) Ensure sample is placed in PBS or PBST for final wash.

6) Refractive index matching/clearing
   a) Submerge sample in 20 mL (can be less for smaller samples) of PROTOS/Optiview/RIMs/FocusClear clearing solution for 24 hrs
   b) Transfer to 25 mL of fresh clearing solution for a further 24 hrs