Tissue Clearing Using CLARITY Method

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1 Works for me
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ABSTRACT

This document covers the CLARITY protocol for volumetric tissue clearing. This method can be applied to many tissue types and is easily modified.

The link below is very useful and should be reviewed before beginning any CLARITY process.
http://wiki.claritytechniques.org/index.php/CLARITY_Technique

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GUIDELINES

General concepts to bear in mind are:

- Larger (>1 cm^3) samples take more time. Use smaller samples until comfortable with the method.
- Keep solutions and tissue cold until the polymerization step. The hydrogel solution contains a heat-activated polymerization initiator, but it is not required. When the hydrogel solution warms up or is undisturbed for long periods of time auto-polymerization will occur, reducing the liquid volume available for tissue perfusion.
- Warmer is better for tissue clearing. Once the sample has been polymerized it is rather hearty. Passive clearing can occur anywhere from room temperature to 60°C. Be mindful however, inadequately perfused/fixed tissue will deteriorate if kept too warm for long periods of time.
- Agitation is good, to a point. Agitation can be achieved with an orbital shaker or multi-axis rocker. For best results use a low intensity setting to avoid tissues impacting container walls.

MATERIALS TEXT

Hydrogel monomer solution (HMS):
(http://wiki.claritytechniques.org/index.php/Solutions)

Ingredient	Amount	Final Concentration	Purpose
40% Acrylamide	40 mL	4%	Hydrogel network monomer
2% Bis-acrylamide	10 mL	0.05%	Small chemical crosslinker
VA-044 Initiator	1 g	0.25%	Polymerization thermal initiator
16% Paraformaldehyde	100 mL	4%	Biomacromolecule crosslinker
10X PBS	40 mL	1X	Salt buffer
Deionized water	210 mL	-	Aqueous solvent

Clearing solution:
Electrophoretic Tissue Clearing Solution, C13001
https://logosbio.com/tissue-clearing_3d-imaging/tissue-clearing/x-clarity

22′-Thiodiethanol **Sigma**

Aldrich Catalog #166782-500G

Equipment:
500 ml beaker
Stir plate and bar
pH meter
Fume hood
50 ml conical tubes
15 ml conical tubes
Long tweezers
Orbital shakers or rocking platforms. Ideally one kept in 4°C, another in 37°C.

SAFETY WARNINGS

Paraformaldehyde and acrylamide are both toxic; therefore, preparation and use of the hydrogel solution should be done in a fume hood.

2,2'-Thiodiethanol (TDE) can cause severe eye irritation. Eye protection and nitrile gloves are recommended for handle this chemical.

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Tissue Clearing using CLARITY method 5d 5h

1 Collect samples into cold 4% PFA

Tissue samples are procured and transferred in cold (4°C) 4% paraformaldehyde (PFA) made with sterile saline or phosphate-buffered saline (PBS) (pH 7.4).

When tissue is received for clearing immediately start step 2 below.

2 CLARITY: Prepare Hydrogel Monomer Solution (HMS) 1h

Prepare hydrogel monomer solution in advance of receiving tissue samples. Recipe for HMS can be found in Materials section.

Prepare HMS in a fume hood. Keep beaker on ice and mix with magnetic stir bar. Finally, adjust pH to 7.4.

Aliquot 40 ml HMS into 50 ml conical tubes and keep at -80°C until needed; thaw at 4°C overnight.

Rule: Keep HMS below 4°C to prevent unwanted auto-polymerization of hydrogel solution.

3 CLARITY: Incubate Samples in HMS 5d

Place sample(s) in cold HMS. Incubate at 4°C under constant gentle agitation to help prevent HMS auto-polymerization.

Rule: Use a sufficient volume of HMS based on the sample size. Generally, a 1 cm^3 block of tissue should incubate in at least 20 ml HMS.

Incubation times may be adjusted based on sample size, density, or species. For example, a 1 cm^3 block of human spleen would be adequately perfused with HMS after 5 days. A mouse spleen of similar size would be perfused after 3 days.

4 CLARITY: Tissue-Hydrogel Polymerization 4h

Fill a 50 ml conical tube with PBS (overfill), secure top, and warm to 37°C.

Use long tweezers to transfer HMS-perfused sample to conical tube containing 37°C PBS. Aim to minimize airspace left in tube. Incubate sample at 37°C with constant gentle agitation for 4 hours.

5 CLARITY: Passive Clearing for Tissue Transparency 4w

Following polymerization samples are transferred to lipid clearing solution. See Materials section for link.

The quality, size, and age of human samples will effect how long it takes for lipids to wash out.

Incubate tissue samples in 50 ml conical tubes filled with clearing solution to 50 ml mark.

Keep samples at 45°C with constant gentle agitation. Clearing solution should be replaced weekly or when pH drops below 8.5.

Sample size is the primary determinant of how long the clearing process will take. Smaller samples will clear quicker.

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6 Assessing Tissue Transparency with Refractive Index Matching

4h

Once sample is visibly clear a final incubation in refractive index matching (RIM) solution should render the tissue almost invisible to the eye.

The refractive index target, in this case n = 1.45, was selected based on imaging system requirements.

Prepare a 63% 2,2'-thiodiethanol (TDE) solution diluted with PBS. Mix the solution gently to avoid creating bubbles. The volume of 63% TDE should be about 10 times the sample volume.

Wash cleared tissue sample in a conical tube with 45 ml PBS, incubating at 37°C with constant gentle agitation for 24 hours.

Add 10 ml of 63% TDE to a 15 ml conical tube and transfer the washed sample. Incubate the sample at room temperature or 37°C with gentle agitation. Once the sample sinks in the RIM solution allow it to incubate another 30 minutes before imaging. Tissue size will effect incubation time.

The tissue should now be fully perfused with RIM solution, optically transparent, and ready to image. Proceed to imaging system to observe quality of clarity throughout the tissue. Regions appearing cloudy are likely not sufficiently cleared or are not completely perfused with RIM solution.

If the tissue is sufficiently cleared then return the sample to a 50 ml conical tube with PBS and wash overnight at 37°C with constant gentle agitation. The cleared tissue is now ready for immunolabeling.

7 Sample Storage

Cleared samples can be kept long term in room temperature clearing solution, or in PBS at 4°C. No agitation is needed.

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