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Tissue Preparation for CLARITY

Seth Currlin[1], Marda Jorgensen[1], Jerelyn Nick[1]

[1]University of Florida

1 Works for me dx.doi.org/10.17504/protocols.io.8jihuke

Optical Clearing of Tissue | Human BioMolecular Atlas Program (HuBMAP) Method Development Community

Jerelyn Nick
University of Florida

ABSTRACT

Tissue preparation for CLARITY includes fixation in 4% PFA, infusion with monomers in Hydrogel Solution, and clearing of lipids in Electrophoretic Tissue Clearing Solution.

EXTERNAL LINK

http://wiki.claritytechniques.org/index.php/Solutions

GUIDELINES

The hydrogel solution components should be kept on ice during preparation to prevent polymerization. The thermal initiator is stable at low temperatures, but initiates polymerization at higher temperatures. To prevent polymerization, the hydrogel solution aliquots should be stored at -20°C until ready for use. However, the solution should be stable at 4°C for a few days and at room temperature for a couple hours.

MATERIALS TEXT

Hydrogel Solution

(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

Hydrogel Solution

Clearing Solution

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

Equipment:
Platform rocker at room temperature (PFA Incubation)
Rocking Stage at 4˚C (hydrogel solution incubation)
Incubator Shaker/ at 37˚C (hydrogel polymerization)
Incubator/Shaker at 45˚C (tissue clearing)

SAFETY WARNINGS

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

1 1. PFA Incubation: Trimmed tissues are placed fixed in 4% PFA for 20-24 hours at room temperature on a slow platform rocker.

2 2. Hydrogel Incubation: Tissues are transferred to Hydrogel Solution in for approximately 5 days at 4˚C. Tubes are kept on a 2-axis rocker for the duration.

3 3. Hydrogel Polymerization: On day 5 the tissue is placed in pre-warmed 37˚C PBS and a layer of mineral oil to deter oxygen exchange from air within tube. The hydrogel within the tissue is polymerized at 37°C for 4 hours with gentle rocking.

4 4. Passive Clearing: Following polymerization the tissue is placed in Clearing Solution and kept at 45˚C with gentle rocking until optically clear.