Protocol: Magnify-Unclearing ExxRM

Logan Thrasher Collins

1. Prepare a monomer solution made of 4% N,N-Dimethylacrylamide (v/v), 34% sodium acrylate (SA) (w/v), 10% acrylamide (AA) (w/v), 0.01% N,N′-Methylenebisacrylamide (Bis) (w/v), and 1% NaCl (w/v) in a chosen volume of 1x PBS before synthesis.

2. Immediately before gelation, add to the monomer solution the following to final concentrations of 0.2% (w/v) APS, 0.2% (v/v) TEMED, 0.001% 4HT (w/v) and 0.25% (v/v) methacrolein. This produces the gelling solution.

3. Prepare an appropriate gelling chamber for the tissue size and geometry. Incubate the sample in the chamber with gelling solution for 30 minutes at 4°C, then incubate the gelling chamber in a humified container overnight at 37°C.

4. Incubate gel with shaking in homogenization buffer at 80°C for 8 hours. Homogenization buffer has the following recipe: 200 mM SDS, 8 M Urea, 25 mM EDTA in a chosen volume of 2x PBS (pH 7.5).

5. Wash the sample 3 times with 1x PBS at room temperature for 10 minutes each.

6. Wash the sample 3 times with 1% PBST at 60°C for 10 minutes each.

7. Wash the sample 3-5 times (or until fully expanded) with pure water at room temperature for 10 minutes each.

8. Pour out excess water and immerse the expanded sample in 50 µM NHS-PEG4-Biotin dissolved in 1x PBS. Rock overnight at 4°C.

9. Wash the gel 5 times with 0.1% PBST for 20 minutes each.

10. Immerse the gel in 1 µg/mL streptavidin-HRP dissolved in 1x PBS. Rock overnight at room temperature.

11. Wash the gel 5 times with 0.1% PBST for 20 minutes each.

12. Wash the gel 3-5 times (or until fully expanded) with pure water at room temperature for 10 minutes each.

13. Pour out excess water and immerse the sample in 3 volumes of EnzMet™ Detect A solution. Incubate for 2 minutes.

14. Add 1 volume of EnzMet™ Detect B solution and agitate to mix. Incubate for 2 minutes.

15. Add 1 volume of EnzMet™ Detect C solution and agitate to mix. Incubate for 10-15 minutes.

16. Wash the gel 3-5 times (or until fully expanded) with pure water + 10% glycerol at room temperature for 10 minutes each. *Note: explore possibility of expanding in glycol methacrylate instead to facilitate post-expansion fixation.*

17. Immobilize the expanded gel in 2% agarose within an appropriate tube for synchrotron x-ray imaging. Seal the tube to prevent evaporation.

18. Image the sample using synchrotron x-ray imaging. Keep at cryogenic temperature during imaging to prevent heating from causing distortions.