HYDROGEL PHANTOMS FOR EXPANSION MICROSCOPY

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Based on the poly-electrolyte gel recipe in Tillberg et al. 2016

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Protocol purpose:
This protocol can be used to prepare fluorescent hydrogel phantoms compatible with expansion microscopy protocols.

PROTOCOL

Step 1 / 5 [Time required 20 minutes]

Preparation of stock solutions

The following stock solutions can be kept for several months in the freezer at -20 °C:

- Prepare a 33 wt% stock solution of sodium acrylate by dissolving 38 g sodium acrylate in 100 mL water. The stock solution should be clear and colorless. If there is a yellow tint to it, discard the bottle and purchase a new one.
- Prepare a stock solution of acrylamide by dissolving 50 g acrylamide in 100 mL water.
- Prepare a stock solution of N,N′-methylenebisacrylamide by dissolving 2 g in 100 mL water.
- Prepare a stock solution of sodium chloride by dissolving 29.2 g acrylamide in 100 mL water.

The remaining solutions can be aliquoted and kept in a freezer at -20 °C for the indicated times:

- Prepare a 0.5% stock solution of inhibitor 4-hydroxy-TEMPO (4HT) (25 mg in 5 mL of water). Can be kept several months at -20 °C.
- Prepare a 10% stock solution of accelerator TEMED. Can be kept several weeks at -20 °C.
- Prepare a 10% stock solution of initiator ammonium persulfate (APS). Can be kept several weeks at -20 °C.

Step 2 [Time required 5 minutes]

Preparation of the monomer solution

To prepare the monomer solution, in a 45 mL conical tube add:

- 2.25 mL of the sodium acrylate stock solution‘
- 0.5 mL of the acrylamide stock solution‘
- 0.75 mL of the N,N′-methylenebisacrylamide stock solution‘
- 4 mL of the sodium chloride stock solution‘
- 1 mL of PBS 10X
- 0.9 mL of water

The final volume is 9.4 mL.

Step 3 [Time required 10 minutes]

Preparation of the slides and gelling chambers

Now would be a good time to start the incubator and set the temperature to 37 °C.

- On a microscope slide, deposit a very small amount of fluorescent beads. Use a finger to make a fingerprint if desirable to create a pattern. The pattern will be useful to assess the expansion factor of the gels under a microscope. One can draw a wide circle around the patterns with a PAP pen (hydrophobic pen) which will help circumscribe the gelling solution on the bead pattern.

EQUIPMENT

fluorescent beads | microscope slides | microscope coverglass # 1 or 1.5 | eppendorf tubes | PAP pen | razor blade | diamond cutter | bucket of ice

histological humidifying chamber | incubator | benchtop vortex

CHEMICALS

Dionized ultrapure water or MilliQ water | Sodium acrylate | Acrylamide | N,N′-methylenebisacrylamide | Sodium chloride | PBS 10X

DANGERS

Chemicals
Physical
Environmental

PROTECTIVE GEAR

Laboratory Coat | Gloves

- With a diamond cutter, cut 2 thin stripes of coverglass which will serve as spacers for the gelling chamber.

Step 4 [Time required 10 minutes]

Preparation of the gelling solution

Generally speaking, 200 μL of gelling solution are needed for the gel embedding of a mouse hemi-brain section. Depending on the desired volume of individual gel phantoms, one can prepare smaller or bigger amounts. The gelling solution should be prepared at the last moment, when one is ready to use it.
For the preparation of 200 μL of gelling solution, mix **on ice** and **in the following order**:

- 188 μL of monomer solution
- 4 μL of 4HT stock solution
- 4 μL of TEMED stock solution
- 4 μL of APS stock solution

Solutions need to be vortexed to ensure full mixing.

Step 4 [Time required 90 minutes]

Gel incubation

- On each fluorescent bead pattern on the slide, dropcast 100-200 μL of gelling solution and form a droplet.
- Build a gel incubation chamber on the microscope slide. The gel chamber is constructed by sandwiching the droplet between the slide and a coverglass with coverglass spacers on either side to prevent compression of the gels. Stacking more spacers on top of the others will make thicker gels. Make sure that the top coverglass sits flat and that there are no air bubbles trapped in the chamber.
- Put the slide in a humidifying box and incubate at 37 °C for 1-1.5 hour. The top of the gel chamber should be easy to remove and the gels should be firm and elastic. Do not let the gels dry.

Step 5 [Time required 45 minutes]

Gel expansion

- Take off the top cover of the gel chamber and submerge the slide in DI water in a petri dish or a slide box or a container of adequate size to accommodate fully expanded gels (3-5X expansion in each direction can be expected). The volume of water has to be minimum 10-fold the final gel volume.
- After 15 minutes, pipette off the water from the petri dish and add fresh DI water.
- Repeat the last steps between 3 and 5 times. The gel expansion will eventually reach a plateau.

SOURCES

References

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WARNINGS

none

NOTES