Fixation for immunocytochemistry for tissue samples.v3

This fixation procedure will allow you to process your samples to a point that they can be store away at -20°C temperature before you decide whether you want to proceed immunocytochemical labeling.

Procedures:

1. Contact Jan A. Redick at the Advanced Microscopy Facility (Tel: (434) 924-2524, email: jar@virginia.edu) to set up the date and time for using the 45°C oven.
2. Euthanize the animals under anesthesia with cervical dislocation.
3. Dissect and harvest the tissue as quickly as possible.
4. Put a few drops of fixative (see below) on wax or plastic petri dish and immediately put the harvested tissue into the fixative. Then cut the tissue specimen into small pieces (1 mm cubes or smaller). For skeletal muscle, it is ideal to have a thin longitudinal strip.

THE FOLLOWING PROCEDURES ARE DONE AT **4°C**

5. Immediately put the sample into 4 ml of ice-cold 4% paraformaldehyde + 0.2% glutaraldehyde in 1X PBS (pH 7.2), in a 5-ml polypropylene tube on ice and gently rotate at 4°C for 1 hour.
6. Wash samples in 2 ml of ice-cold 1X PBS 3 x 10 min with rocking.
7. Wash samples in 2 ml of ice-cold 40% ETOH for 10 min with rocking.
8. Wash samples in 2 ml of ice-cold 60% ETOH for 10 min with rocking.
9. Wash samples in 2 ml of ice-cold 80% ETOH for 10 min with rocking.
10. Wash samples in 2 ml of ice-cold 100% ETOH for 2 x 10 min with rocking.

THE FOLLOWING PROCEDURES ARE DONE AT **ROOM TEMPERATURE**

11. Incubate (with rocking) the samples in 2 ml of 2:1 100% ETOH:LRW for ½ -1 hour.
12. Incubate (with rocking) the samples in 2 ml of 1:1 100% ETOH:LRW for 2-3 hrs (or overnight) (OK stopping point for convenience)
13. Incubate (with rocking) the samples in 2 ml of 1:2 100% ETOH:LRW for 2 hrs (or overnight) (OK stopping point for convenience)
14. Incubate (with rocking) the samples in 2 ml of 1:4 100% ETOH:LRW for 2 hrs (or overnight) (OK stopping point for convenience)
15. Incubate (with rocking) the samples in 2 ml of 100% LRW for 1-2 hrs (or overnight) (OK stopping point for convenience)
16. Incubate (with rocking) the samples in 2 ml of 100% LRW for 2-3 hrs.
17. Incubate (with rocking) the samples in 2 ml of 100% LRW for 2-3 hrs.
18. Embed the samples in gelatin capsules (not in BEEM capsules or Eppendorf tubes): First, write labels in pencil (not pen or printer ink), and put labels inside capsule, near the top. Fill capsule to top with fresh LRW. Add tissue and let sink to bottom. Push capsule tops on snugly (NOTE: Oxygen will inhibit polymerization).
19. Polymerize the samples in capsule for 24 hours in a 45˚C oven. (Note: do not polymerize at the usual 60˚C...too hard on antigens)

Solution preparation:

8% paraformaldehyde in 1X PBS

1. Wear gloves, goat and goggles.

2. Add 70 ml of ddH2O into a 250-ml beaker.
3. Add 8 g paraformaldehyde and mix them on a stir plate in a fume hood.
4. Add NaOH (~13-15 pellets) to bring pH to ~12 and wait until the paraformaldehyde powder gets completely dissolved.
5. Add 10 ml of 10X PBS, pH 7.2.
6. Add 10N HCl to bring pH back to ~8.0 and then use 1N HCl to bring it to 7.2.
7. Double check pH to make sure it is 7.2 (confirm with pH paper)
8. Add about 10 ml ddH2O to a final volume of 100 ml.
9. Filter sterilize the solution and store at 4°C up to 1 month.

0.4% Glutaraldehyde in 1X PBS

1. Add 5 ml of 10X PBS, pH 7.2, to 50 ml conical tube.
2. Add 44.2 ml of ddH2O.
3. Add 0.8 ml of 25% glutaraldehyde* to a final volume of 50 ml.
4. Make this solution fresh for each use.

* Pippette up and down the glutaraldehyde to ensure the solution is homogenous before adding.

4% paraformaldehyde, 0.2% glutaraldehyde in 1X PBS

Mix 8% paraformaldehyde in 1X PBS with 0.4% glutaraldehyde in 1X PBS 1:1 to make the final solution. This solution should be made fresh for each use. Label it "For immunogold".

ETOH:LWR mix

Make this fresh before use.