

Fixation for regular TEM.v2

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 sample processing.
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 (see below)**. 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.
5. Immediately **put the sample into 4 ml of ice-cold 4% paraformaldehyde + 2.5% glutaraldehyde in 0.1 M NaCacodylate (pH 7.2-7.4)**, in a polypropylene tube. Rotate the tube(s) at 4°C for 4 hours or overnight before storing it at 4°C for up to a few months or submitting them to EM facility.

Solution preparation:

8% paraformaldehyde in 0.1 M NaCacodylate

1. Wear gloves, goat and goggles.
2. Add 30 ml of ddH₂O and 40 ml of 0.2M Na Cacodylate buffer pH 7.4 (EMS Cat#11650) into a 250-ml beaker.
3. Add 8 g paraformaldehyde and mix 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 10N HCl to bring pH back to ~8.0 and then use 1N HCl to bring it to 7.2-7.4.
6. Add 10 ml of 0.2 M NaCacodylate buffer.
7. Double check the pH to make sure it is 7.2-7.4.
8. Add about 10 ml of ddH₂O to a final volume of 100 ml.
9. Filter sterilize the solution and store at 4°C up to 1 month.

5% glutaraldehyde in 0.1 M NaCacodylate

1. Add 25 ml of 0.2 M NaCacodylate buffer, pH 7.4 (EMS Cat#11650) to a 50-ml conical tube.
2. Add 15 ml of ddH₂O.
3. Add 10 ml of 25% glutaldehyde* 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, 2.5% glutaraldehyde in 0.1 M NaCacodylate

1. Mix 8% paraformaldehyde in 0.1 M NaCacodylate with 5% glutaraldehyde in 0.1 M NaCacodylate 1:1 to make the final solution. This solution should be made fresh for each use. Label it "**For TEM**".