

TEM FIXATION PROTOCOLS:

Chemicals needed (will vary depending on sample): fixative – (25%, 50%, or 70%) glutaraldehyde, or 16% paraformaldehyde; post-fixative – 4% osmium tetroxide; wash buffer (e.g. Sorensen's, Sodium Cacodylate, PBS); deionized water (DH₂O) acetone or ethanol dehydration series; embedding resin (e.g. Spurr's, London Resin, Epon)

Wash Buffers:

0.2M Sodium Cacodylate Buffer:

- 4.28g Sodium Cacodylate
- 100 mL DH₂O
- Adjust pH as needed for sample.

0.2M Sorensen's Buffer:

- 25 mL Sorensen's Buffer (purchased from EMS- Ft. Washington, PA in premixed form or see recipe below)
- 25 mL DH₂O (Note: one can dilute this buffer with DH₂O further if needed.)
- Adjust pH to 7.0 or 7.2

Recipe:

- pH 7.2: Add 72 mL 0.2M Dibasic Sodium Phosphate (35.61 g/L) to 28 mL 0.2M Monobasic Sodium Phosphate (31.21 g/L)
- pH 7.4: Add 81 mL 0.2M Dibasic Sodium Phosphate (35.61 g/L) to 19 mL 0.2M Monobasic Sodium Phosphate (31.21 g/L)

Fixatives:

2.5% Glutaraldehyde in 0.1M Buffer:

- 10 mL 25% Glutaraldehyde
- 50 mL 0.2M Sorensen's or Cacodylate buffer at required pH
- 40 mL DH₂O

Make fresh before use and store at 4C – use within 2 weeks.

0.5% Glutaraldehyde in Sorensen's Buffer:

- 0.5 mL 50% Glutaraldehyde
- 5 mL Sorensen's Buffer (purchased in bottle)
- 44 mL DH₂O

Make fresh before use and store at 4C – use within 2 weeks

1.6% Paraformaldehyde and 1% Glutaraldehyde in 0.1M Buffer:

- 5 mL 16% Paraformaldehyde
- 3.2 mL 25% Glutaraldehyde
- 40 mL 0.2M Sorensen's or Cacodylate Buffer at required pH
- 31.8 mL DH₂O

Make fresh before use and store at 4C – use within 2 weeks

Post-Fixative:**1% Osmium Tetroxide:**

- 0.1g OsO₄ Crystals
- 10 mL DH₂O
- or
- 2 mL 4% OsO₄
- 4 mL Sorensen's Buffer
- 2 mL DH₂O (can substitute buffer if desired)

Dehydration Series:

Ethanol: 10%, 30%, 50%, 70%, 90%, 100%, 100% in Zeolite beads (molecular sieve)

Acetone: 10%, 30%, 50%, 70%, 90%, 100%, 100% in Zeolite beads (molecular sieve)

Protocol (derivations exist depending on your sample):

1. Under a hood and while wearing gloves, place fixative of choice (either 0.5% or 2.5% glutaraldehyde fix or 1% paraformaldehyde/1%glutaraldehyde fix) into as many glass scintillation vials (with cap) as needed, and place on ice to chill for 5 min.
2. Place sample(s) to be fixed into each vial, and keep on ice for 30-60 min (can be longer depending on sample). If desired, place tubes in vacuum desiccator and apply vacuum- this can release air from sample and improve fixation.
3. Gently mix the sample every 5-10 min. Meanwhile, put the post-fixative (OsO₄) and wash buffer on ice to cool.
4. Remove the fixative using a pipette and discard into waste container. Be careful not to remove any specimen(s) while doing so.
5. Wash the samples with the wash buffer 3x for 5min each, keep samples on ice during this time. Remove the wash with pipette as above.
6. After the last wash, add a small amount of the OsO₄ post-fixative to the samples (just enough to cover). Gently mix and keep on ice for 2hr.
7. Repeat step 4 & 5, and allow samples to come to room temperature.
8. Begin the dehydration series of acetone or ethanol, depending on your embedding resin of choice.
Generally Spurr's = acetone, London Resin = ethanol
Ethanol: 10%, 30%, 50%, 70%, 90%, 100%, 100% in Zeolite beads (molecular sieve)
Acetone: 10%, 30%, 50%, 70%, 90%, 100%, 100% in Zeolite beads (molecular sieve)
 - a. Remove the wash and add 10%. Gently mix and let sit for 20-30 min.
 - b. Remove the 10%, discard in waste, and add 30%, gently mix and let sit for 20-30 min.
 - c. Repeat this step up to 100% in zeolite beads (If needed, the samples can sit overnight in 70% acetone or ethanol)

- 9.** Prepare the embedding resin according to manufacturer instructions. Make small aliquots of the following ratios:

Spurr's:

1. Spurr's/acetone: 25/75

2. Spurr's/acetone: 50/50

3. Spurr's/acetone: 75/25

4. Spurr's – 100%

London Resin:

LR/ethanol: 25/75

LR/ethanol: 50/50

LR/ethanol: 75/25

LR- 100%

- 10.** Replace the 100% acetone (or ethanol) with the first embedding step mixture. Gently swirl the sample in the solution and let sit for 2 hrs – at RT for Spurr's, 4C for London Resin.
- 11.** Repeat this with the remaining concentrations as listed above in step 9.
- 12.** Place sample into 100% resin (Spurr's or LR) and let sit overnight (at RT or 4C).
- 13.** Place samples into molds, Beem capsules, or other container, and polymerize according to manufacturer instructions.