

TEM MONOLAYER PROTOCOL

Gloves must be worn throughout this procedure. All work must be done in a fume hood.

Use for very small pellets or if sample is in a plastic container.

- Rinse cell monolayers briefly in 0.01M PBS.
- Fix in situ with 2.5% glutaraldehyde in 0.01M PBS, pH 7.4, for 1 hour at room temperature.
- Wash 3 times in 0.01M PBS buffer (10 minutes each).
- Post-fix monolayers for 1 hour at 4° C in 1% OsO₄ with 1% potassium ferricyanide.
- Wash 3 times in 0.01M PBS buffer (10 minutes each).
- Dehydrate monolayers in a graded series of alcohol (30%, 50%, 70%, and 90% - 10 minutes) with three changes in 100% ethanol (15 minutes each).
- Change three times in epon (1 hour each).
- Remove last change of epon and invert beam capsules full of resin over relevant areas of the monolayers and polymerize at 37° C overnight and then 48 hours at 60° C.
- Pop off the beam capsules and underlying cells from the bottom of the petri dish and section.