EM Fixation Protocol 02 - Conventional TEM Sample Preparation of Cell

Cell monolayer from cell lines or primary cells

Note: Cells should be seeded, cultured, and attached on an 8mm diameter Thermanox coverslip in a 12 well culture plate before fixation.

Purpose of Fixation

- Prevention of autolysis
- Protection from damage

Check List

- ✓ 2.5% glutaraldehyde (GA) in 0.1M sodium cacodylate buffer (CB) from Microscopy Core
- ✓ 8mm diameter Thermanox (TMX) coverslip from Microscopy Core
- ✓ 12 well culture plate from Microscopy Core
- ✓ Parafilm

Cell Fixation

1. No rinse required before cell fixation.
2. Warm up the 2.5% GA fixative in water bath or incubator at 37 °C.
3. Add the warm 2.5% GA fixative of same volume as cell incubation medium along the wall of the 12 well culture plate (vol of fixative/vol of medium = 1/1).
4. Swivel the culture plate 4-5 times in the culture bench.
5. Leave the culture plate for 5 minutes in the culture bench.
6. Label with clear sample identification number
7. Seal well the culture plate with parafilm.
8. Keep the sealed culture plate at 4 °C until your conventional TEM sample preparation training/service is scheduled.

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