

## EM Fixation Protocol 03 - Conventional TEM Sample Preparation of Tissue

### Soft organ tissue from small animal brain, heart, liver, lung and kidney

#### Purpose of Fixation

- Prevention of autolysis
- Insolubilization of tissue
- Protection from putrefaction
- Protection from damage

#### Check List

- ✓ 3% glutaraldehyde (GA) + 3% paraformaldehyde (PFA) in 0.1M sodium cacodylate buffer (CB)
- ✓ Parafilm
- ✓ 20ml Glass scintillation vial
- ✓ Transfer pipette
- ✓ Double-sided razor blade
- ✓ 4ml Glass sample vial
- ✓ Cotton swab
- ✓ Weighing dish

#### Organ Fixation

**Note: Fast fixation of the organ is extremely important to prevent autolysis.**

1. Briefly rinse the organ from animal with 1X PBS to remove blood.
2. Put the organ into the 20ml glass scintillation vial filled with 5 - 10 ml of 3% GA + 3% PFA in 0.1M CB (w/v = 1/5).
3. Leave the organ into the scintillation vial in fume hood for 1 hour for initial fixation.

#### Tissue Dissection

**Note: Strongly recommend doing on parafilm with a drop of ice-cold 3% GA + 3% PFA in 0.1M CB and the dissected tissue should be wet with the fixative always.**

4. Make the organ to 1-2 mm<sup>3</sup> size of tissue sample without physical pressure and damage by slicing with crossover of two half-razor blades. If a sample's orientation is required, make the organ to 1 x 1 x 4 mm size of tissue sample.

**Note: Make 6 – 7 cubes with clear side of slice by one slicing only.**

5. Put them carefully into the 4ml glass sample vial filled with 1ml of ice-cold conventional TEM fixative (w/v = 1/10) by touching with a cotton swab wet with the conventional TEM fixative.
6. Label with clear sample identification number or name.
7. Keep the labeled sample vial at 4 °C until your conventional TEM sample preparation training/service is scheduled.