

## Instructions for TEM fixative kit

### Materials in set:

1. ampule: 8% glutaraldehyde, 10ml (must be stored at 4°C)
  2. ampule: 10% paraformaldehyde, 10ml
  3. plastic vial: 0.2M Na cacodylate buffer ("2X"), 20 ml for dilution of aldehydes.
  4. plastic vial: 0.1M Na cacodylate buffer ("1X"), 20 ml for buffer wash.
- Other supplies: beaker for mixing, razor blades, cutting dish, plastic transfer pipette, shell vials for sample storage.

### Fixative preparation:

Just before use, mix the 20 mls of 0.2M Na cacodylate buffer and the contents of both ampules.

This makes 40 mls of Karnovsky's fixative: 2% glutaraldehyde, 2.5% formaldehyde in 0.1M sodium cacodylate buffer.

### An example fixation method:

1. Cover fresh tissue with a droplet of freshly mixed fixative then mince with razor blades until tissue segments are less than 1 mm. (i.e., 1 mm cube or 1 mm thick slices).
2. Transfer tissue (without crushing) to a sample vial with at least 10 volumes of fresh fixative solution
3. Fix for 1 hour at room temperature then overnight at 4C.
4. Deliver to VTPB electron microscopy lab

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