

**Preparation of 4% paraformaldehyde 2.5%glutaraldehyde fixative.**

**PREPARATION MUST BE CARRIED OUT IN THE FUMEHOOD. WEAR GLOVES!**

**Materials**

1. 4g Paraformaldehyde powder
2. 10ml 25% glutaraldehyde
3. 1M NaOH
4. 50ml dH<sub>2</sub>O
5. 50ml 0.1M phosphate buffer pH 7.2

**Method**

1. Weigh paraformaldehyde powder (4g) into a 30 ml conical (Falcon) tube with a screw top.
2. Add 30 ml dH<sub>2</sub>O.
3. Heat to approximately 70°C (screw cap on loose) in a beaker of water on a hotplate (check the temperature regularly – do not let it boil).
4. Once at 70°C the powder will start to dissolve over 10-15 mins, carefully add 1-4 drops 1M NaOH until the solution becomes clear. *DO NOT add too much as this will raise the pH too much.*
5. Place the tube on ice and allow to cool to room temperature.
6. Add 10ml of glutaraldehyde (25% stock solution in dH<sub>2</sub>O) and make up to 50ml with distilled water.
7. Mix equal volumes of glut/paraformaldehyde and 0.1M phosphate buffer. Check pH 7.2 (might need slight adjustment with NaOH). Keep at 4°C until use.

**Notes**

The fixative should be made fresh and discarded after a week as self-polymerisation will occur in solutions stored for extended times.