Standard operating procedure (SOP) for preparing 4% PARAFORMALDEHYDE solution (PFA)

Paraformaldehyde is used for the fixation of brains or tissue.

**PARAFORMALDEHYDE** (white crystalline powder, storage temperature: 2-8 °C)

- Flammable.
- Toxic by inhalation, harmful by ingestion, irritant, skin sensitiser.
- Paraformaldehyde has been listed as a **HAZARDOUS SUBSTANCE according to the Safe Work Australia Criteria and as a DANGEROUS GOOD by the Criteria of the ADG code**.
- This product presents a moderate hazard with normal use.
- Harmful to aquatic life. Do not let product enter drains. Discharge into the environment must be avoided.

**SODIUM HYDROXIDE (NaOH)** (white pellets)

- Corrosive to the eyes, skin, and respiratory tract; do not breathe dust. Material is extremely destructive to the tissue of the mucous membranes, skin and upper respiratory tract.
- This product has the potential to cause serious adverse health effects. Use safe work practices to avoid eye or skin contact and inhalation. Over exposure may result in severe burns with corrosive tissue damage. Upon dilution, the potential for corrosive effects may be reduced.
- Sodium hydroxide has been listed as a **HAZARDOUS SUBSTANCE according to the Safe Work Australia Criteria and as a DANGEROUS GOOD by the Criteria of the ADG code**.
- This product presents a High hazard with normal use
- Discharge into the environment must be avoided, harmful to aquatic life.

**Materials:**

- dH₂O - distilled water
- Paraformaldehyde
- Potassium Phosphate Monobasic (KH₂PO₄)
- Sodium Chloride (NaCl)
- Sodium Hydroxide (NaOH) pellets
- Sodium Phosphate Dibasic (Na₂HPO₄·7H₂O)
Procedure:

- You must be wearing a lab coat, gloves, face mask and eye protection.
- The sash on the fume hood should always be set as low as possible.

To make 1 liter of 4% paraformaldehyde (PFA) (this makes enough for 4 rats):

STEPS 1-5 MUST BE DONE IN THE FUME HOOD

1. Heat 600 mls of distilled water in a glass beaker to 40-50°C
2. Carefully weigh out (in the fume hood) 40g of paraformaldehyde powder.
3. Slowly add the powder to the water (watch temp and adjust the hot plate accordingly so it remains at 40 C).
4. It usually takes 1-2 hrs for the PFA to dissolve. (please note that the PFA will not completely dissolve, which is fine).
5. Once dissolved, add one Na OH pellet at a time until the solution becomes clear. TAKE CAUTION WHEN ADDING PELLETS, DO NOT TOUCH WITH YOUR HAND!
6. Cover the beaker with foil and place the cleared solution in the refrigerator for approximately 1 hr to cool before filtering.

STEPS 7-10 MUST BE DONE IN THE FUME HOOD

7. Filter through whatman #1 paper and a funnel. If available use a filter pump to expedite the process.
8. To the filtered solution add 3.4 g of potassium phosphate monobasic, 10.9 g of sodium phosphate dibasic and 9 gm of NaCl and stir until dissolved.
9. Pour into a 1L graduated cylinder and fill with distilled water to the 1 L (1000ml line).
10. Pour into glass bottle with blue screw top lid- be sure this bottle is clearly labeled.

Disposal:

1. Carefully decant waste materials (this includes Blood/PFA waste) in designated chemical containers.
2. Containers must be closed and labeled “hazardous waste” along with the details of main constituents.
3. Place waste in waste collection area (located under the bench on the left hand side as you walk into the surgery room).