# OLSON LAB PROTOCOL: 4% Paraformaldehyde (PFA) in Phosphate-Buffered Saline (PBS)

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**NOTES**

- Fixative for immunocytochemistry of fresh tissues (e.g. WMISH)
- PFA cross-links the biomolecules of the cell, acting as scaffolding that anchors the mRNA whilst sill allowing it to hybridise with complimentary strands of RNA (i.e. riboprobes).
- PBS buffers the tissues from enzymatic damage during fixation
- It is essential to use fresh PFA (< 1 month old when stored refridgerated) in order to acheive good cellular level fixation, and thus good WMISH results (N.B. PFA breaks down quickly in solution)
- Also important to avoid over-fixation as this over cross-links the biomolecules preventing hybridisation whilst also increasing auto-fluorescence (which increases background ‚noise' when using fluorescent probes)
- Fix tissues in PFA in a refridgerator overnight (not longer), then proceed to WMISH or dehydrate in a graded ethanol series and store indefinitely at -20.
- **Prepare 4% PFA in a fume hood**
- **Wear gloves and avoid fumes and air-borne powder**

**Preparation from Ampules of 16% Stock Formaldehyde Solution:**

1. Add one 5 ml or 10 ml ampule to 3 equal parts of PBS or PBSAT. Use immediately and/or keep refridgerated up to 1 week. (N.B. Although fast and convenient, PFA mixed from ampules is *exceedingly* more expensive than that prepared from powder)

**Preparation from Powdered PFA (final volume = 500 ml):**

- Put a stirrer/hot plate in the fume hood in the lab (borrow from prep lab)
- Use a 500 ml beaker
- Add a magnetic stir bar and a thermometer to monitor temperature throughout

1. Add 20 g powdered PFA to 250 ml DEPC-treated ddH2O (powder will not dissolve)

2. Heat and stir mixture on hot plate slowly but **keep temperature below 60°C*** (optimal is 55-57°C). Solution will look cloudy. (N.B. temperatures above 60 C will denature the PFA, making it useless as a fixative)

3. PFA will start to dissolve slowly, typically over a period of 20-60 mintues

4. Add drops of 1M NaOH (found in prep room) while stirring continuously until all PFA is dissolved (if necessary).

5. Let solution cool down and mix with 250 ml (or 1 part) of 0.3M PBS (pH 7.2) or PBSAT

6. Move solution to the prep room and adjust pH to 7.2-7.3 using drops of HCl (while stirring)

7. Pour into 500 ml glass jar with screwcap, **label with name, concentration and date**, and keep in refridgerator for up to 1 month. Alternatively, aloquot into 10 x 50 ml falcon tubes, label and date and freeze at -20 C. Frozen aloquots are good for at least 5 years.

**Preparation of Phosphate-buffered saline (PBS) / PBS with Tween-20 (PBSAT):**

**using PBS tablets (makes 1L):**

1. Add 5 (Sigma P4417) or 10 (Oxoid BR0014) PBS tablets to 1 L DEPC-treated water.
2. Autoclave. After cooling, add 1 ml Tween-20 (optional)*

**using reagents (makes 250 ml):**

0.3 M PBS make up 250ml with $ddH_20$

| | |
|---|---|
| NaCl..... | 4.25 g |
| $NaH_2PO_4 . 2H_2O$ (0.012M)..... | 1.95g |
| $Na_2HPO_4 . 12H_2O$ (0.038M)..... | 13.5g |

(or use sodium phosphate with less $H_20$, but calculate molarity )

Final pH = 7.2

*N.B. Tween-20 acts as a lubricant and is added to reagents used for WMISH to prevent specimens from sticking together during processing