

## Preparation of Paraformaldehyde Fix

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**NOTE:** For use with RNA protocols, prepare with DEPC-treated water – otherwise, use regular DH<sub>2</sub>O

### Paraformaldehyde fix

2 g paraformaldehyde  
25 ml DEPC water  
5 ml 10X PBS  
20 ml DEPC water

- a) Heat 25 ml DEPC water in a 100 ml sterile beaker (can be RNase zapped) with vigorous stirring.
- b) Weigh out paraformaldehyde making sure to wear a mask and use gloves.
- c) Add paraformaldehyde to fast stirring, heated water – stir for about a minute, do not allow to boil. Powder will not dissolve.
- d) Remove from heat and continue to stir.
- e) Add 1 – 2 drops of 1 N NaOH to solution. The solution should clear.
- f) Once clear, filter the solution through a sterile filter and check that volume is 25 ml – if not add up to 25 with DEPC water.
- g) Add 5 ml of 10X PBS and 20 ml of additional DEPC water.
- h) Check pH – should be around 7.0 – 7.5. If not start over.

Store in Coplin jar

Make up fresh every couple of days. Store in fridge

### 10X PBS

Make up in 800 ml	800 ml
0.02M NaH <sub>2</sub> PO <sub>4</sub> (monobasic) 120 g/m	1.92
0.077M Na <sub>2</sub> HPO <sub>4</sub> (dibasic) 142 g/m	8.8
1.4 M NaCl 58 g/m	69.6
add water to 800 ml	
DEPC treat with 0.8 ml DEPC or DMPC overnight and then autoclave	