Preparation of Paraformaldehyde Fix

Protocol author: Virginia Weis
Date: 2002

**NOTE:** For use with RNA protocols, prepare with DEPC-treated water – otherwise, use regular DH$_2$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$_2$PO$_4$ (monobasic) 120 g/m 1.92  
0.077M Na$_2$HPO$_4$ (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