

# **Fixation of Embryonic Tissue: Antibody Staining of Transcription Factors on Frozen Sections.**

## **0.2M Phosphate Buffer (P.B) pH7.2**

16.6 gr  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$   
2.5 gr  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$   
water to 400 ml

## **4% paraformaldehyde (in 0.1MPB) (make fresh)**

50 ml water  
4 gr paraformaldehyde  
heat to 60-70 C, add 1 drop 10 N NaOH, and stir 10 minutes to dissolve  
add 50 ml 0.2M P.B. (final conc. 0.1M)  
sterile filter and store on ice up to 1 day

## **30% sucrose in 0.1M P.B. solution**

100 ml 0.2M P.B.  
60 gr sucrose  
dissolve and bring final volume up to 200 ml with water

## **Procedure**

When fixing a monolayer of cells, add ice cold 4% paraformaldehyde for 10 minutes. The morphology of the cells can be improved by using 0.12M PB in the 4% PF. Then wash 3X with PBS before adding primary antibody.

Tissue should be fixed with ice cold 4% paraformaldehyde (in 0.1MPB pH7.2). The fixation time should be short: 45 minutes to 2 hours depending on the age of the embryo (ie. E14 mouse and E6 chick fixation times are 1.5 hours. Shorter fixation times for younger animals). The guts should be removed from chick E4 or older and mouse E14 or older if you are looking at neural tube. After fixation it is important to wash the tissue extensively in cold PBS: 3, 50 ml changes of PBS followed by at least 1 hour in PBS on ice. Then transfer the tissue to cold 30% sucrose in 0.1MPB, pH7.2 and allow it to equilibrate overnight at 4°C.

Postnatal and adult animals should be perfused. After perfusion, the tissue of interest should be dissected out and fixation should be continued for an additional 2 hours. Then continue as above.

Note: Primary antibodies (especially those against internal structures) are incubated in 0.1% TX100 overnight at 4°C. Secondary antibodies are incubated for 30 min at RT.