

## Fixation Protocols

### METHANOL FIX:

1. Remove media and wash cells with 1x PBS.
2. **GENTLY** and **SLOWLY** add ice-cold Methanol (stored at -20°C)
3. Store slides with methanol in -20°C freezer for at least 10 minutes.  
(Coverslips can be stored in methanol in freezer for extended times when plates are wrapped in parafilm to prevent evaporation).
4. Remove 50% of the methanol and add 1x PBS **Slowly** back to the original volume. Repeat this 4-5 times to slowly rehydrate sample.
5. Then **Slowly** wash entire volume with 1x PBS

### PARAFORMALDEHYDE FIX:

1. Remove media and wash cells with 1X PBS.
2. Slowly add 4% paraformaldehyde (PFA, made with 1X PBS; Fischer Scientific cat. AA433689M) to cells for 20 minutes at room temperature.
3. Wash cells 3 times with 1X PBS.
4. Quench cells with 50mM NH<sub>4</sub>Cl (ammonium chloride; Fisher cat. A661-500) for 15 minutes.
5. Wash cells 3 times with 1X PBS.
6. Permeabilize cells with 0.1% Triton X-100 (Fisher cat. BP151500) for 5 minutes.
7. Wash cells 3 times with 1X PBS.

### FIX/PERM PARAFORMALDEHYDE/TRITON-X100:

1. Remove media and wash cells with 1X PBS.

2. Slowly add 4% paraformaldehyde (PFA, made with PBS) + 0.5% Triton-X100 to cells for 20-30 minutes at room temperature.
3. Wash cells 3X with 1X PBS.
4. Quench cells with 50mM NH<sub>4</sub>Cl (ammonium chloride) for 15 minutes.
5. Wash cells 3X with 1X PBS.

## Reagents

### 10X PBS

(1.37M NaCl, 27mM KCl, 100mM Na<sub>2</sub>HPO<sub>4</sub>, 18mM KH<sub>2</sub>PO<sub>4</sub>)  
160g NaCl, 4g KCl, 54.4g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O, 4.8g KH<sub>2</sub>PO<sub>4</sub>, add to 1600ml dH<sub>2</sub>O, pH to 7.4, bring to final volume of 2L, store at room temp

### 1X PBS

(137mM NaCl, 2.7mM KCl, 10mM Na<sub>2</sub>HPO<sub>4</sub>, 1.8mM KH<sub>2</sub>PO<sub>4</sub>)  
Dilute 10X PBS 1:10 w/dH<sub>2</sub>O

### 4% PFA

(4% paraformaldehyde in 1X PBS)  
Place 4g PFA and 100ml 1X PBS in a 250ml Erlenmeyer Flask with a stir bar. Make a stopper by twisting kimwipes and place in the mouth of the flask. Heat slowly to boiling in a fume hood so that the PFA is fully dissolved. Good for 2 weeks at room temp.

### 50mM NH<sub>4</sub>Cl

0.267g NH<sub>4</sub>Cl dissolve in 80ml dH<sub>2</sub>O, bring to 100 ml final volume, store at

### 20% Triton X-100

10mL Triton x-100/50ml dH<sub>2</sub>O and filter sterilize (Fisher Scientific, 09-741-88)