

# IMMUNOHISTOCHEMISTRY SOLUTIONS

## Phosphate Buffers

### 0.2M Phosphate Buffer (PB), pH 7.40

Na <sub>2</sub> HPO <sub>4</sub> *Dibasic	23g
NaH <sub>2</sub> PO <sub>4</sub> *Monohydrate	5.2444g
dH <sub>2</sub> O	To 1000 mL

### 0.1M Phosphate Buffer, pH 7.40

0.2M PB	500mL
dH <sub>2</sub> O	500mL

### 0.1M Phosphate Buffer with Azide, pH 7.40

NaN <sub>3</sub> (sodium azide)	0.05g
0.1M PB	To 500mL

### 0.5M Phosphate Buffer, pH 7.40

Sodium Dibasic Phosphate	67g in 500mL dH <sub>2</sub> O (and heat)
Sodium Monobasic Monohydrate	6.9g In 100mL dH <sub>2</sub> O

## PFA's

### 4% Paraformaldehyde, pH 7.40

Paraformaldehyde	20g in 400mL dH <sub>2</sub> O
dH <sub>2</sub> O	To 500mL

Wrap beaker with parafilm in chemical hood  
Heat to ~50°C with a stir bar rotating  
**Do not allow** heat to go above 60°C  
NaOH tablets can be added to help clear the solution  
Vacuum filter and pH to 7.40

### 11.43% PFA, pH 7.40

Paraformaldehyde	57.2g in 400mL dH <sub>2</sub> O
dH <sub>2</sub> O	To 500mL

Wrap beaker with parafilm in chemical hood  
Heat to ~50°C with a stir bar rotating  
**Do not allow** heat to go above 60°C  
NaOH tablets can be added to help clear the solution  
Vacuum filter and pH to 7.40

### Alternative 4% PFA method, pH 7.40

11.43% PFA	175mL
Picric Acid	75mL
0.2M PB	250mL

This method does not require heating, but does require a chemical hood and gloves.

### FOR ALL PFA SOLUTIONS (IMPORTANT)

All steps of PFA solution making **must be done** under a functioning chemical fume hood while wearing gloves to avoid exposure.  
All pH measurements should be done with **pH strips**, *not* the pH meter.

