

## Paraformaldehyde Fixation for Immunofluorescence Microscopy

### • Recipes

#### 37% PFA solution

1.85 g paraformaldehyde  
3.5 ml dH<sub>2</sub>O  
10 µl 10M KOH

- mix together in Falcon tube
- heat in 65° water bath swirling frequently to mix until PFA goes into solution

#### Fixation Buffer (prepare fresh)

3.7% paraformaldehyde solution in phosphate buffered saline (PBS)

### • Paraformaldehyde fixation for non-adherent cells

- coat coverslips with an excess of 0.01% poly-L-lysine for 10 minutes
- aspirate poly-L-lysine solution and dry coverslips completely
- transfer cells in medium to 50 ml tubes
- centrifuge at 400g for 5 minutes
- aspirate the medium and resuspend the cells in PBS
- cover the dried, treated coverslips with the cell suspension
- incubate for 30-60 minutes
- aspirate excess cell suspension
- rinse briefly in PBS
- flood with excess of 3.7% PFA for 10 to 15 minutes
- remove PFA and wash three times with PBS

### • Paraformaldehyde fixation for adherent cells

- grow adherent cells on cover slip
- when subconfluent, wash cells with PBS
- flood with excess of 3.7% PFA
- incubate in PFA for 10 to 15 minutes
- remove PFA and wash three times with PBS