Alternative Fixation with paraformaldehyde

Solution A – 100mM Sodium phosphate, pH 7.4, 30ml

Stock 1M dibasic Na-phosphate, pH 9, 100ml
Stock 1M monobasic Na-phosphate, pH 4.1, 100ml
Stock 100mM Sodium phosphate, pH 7.4, 30ml

- 1M dibasic Na-phosphate 2430 ul
- 1M monobasic Na-phosphate 570 ul
- dH_2O 27 ml

mix two solutions in the given proportions (19:81) to get a pH of 7.4

Solution B – 8% paraformaldehyde, 100 ml (+10 ml NaCl)

Solubilize at room temperature by adding 8 g of paraformaldehyde to 95 ml of 10 mM NaOH. Stir several hours until solution clears. The pH starts at about 12.5 and drops as the paraformaldehyd dissolves. I wait until it drops around pH 10. Set the pH back to about 7.4 with HCl, and adjust the volume to 100ml with dH_2O. Note that some procedure recommend heating but paraformaldehyd decomposes into formaldehyd at 60^0C. Filter through 0.22um filter.

Prepare:

1. Solution A Prepare 100mM sodium Phosphate , Ph 7.4 , 30ml
2. Solution B Prepare 8% paraformaldehyde stock solution
3. combine Solution A 3 parts and Solution B 1 part
4. Prepare PBS + 3% BSA + 1% Saponin + 0,05% Na Azide, Filter
5. Prepare PBS + 1% Saponin.

1. Grow cells on slides
2. Wash 1x with PBS
3. Fix with Fixative for 10-15 min at RT
4. Wash 3 times with PBS (you can leave it in PBS overnight 4 degrees)
5. Permeabilization and blocking with PBS + 3% BSA + 0.1% Triton X-100 + 1% Saponin + 0,05% Na Azide for 10min at RT
6. Incubation primary antibody 1-4hrs at RT in a humified chamber in PBS + 3% BSA + 1% Saponin+ 0,05% Na Azide
7. Wash 3 times with PBS + 3% BSA + 1 % Saponin+ 0,05% Na Azide
8. Incubation secondary antibody 30min-1hr with PBS + 3% BSA + 1 % Saponin + 0,05% Na Azide at RT in the dark
9. Wash 3x PBS + 1 % Saponin
10. Mount coverslip and keep in the dar