

## Cell Fixation Procedure:

### Materials:

*Phosphate buffered saline supplemented with (0.1g/L CaCl<sub>2</sub>) and (0.1g/L MgCl<sub>2</sub>), referred to as PBS++ in the document. Store at 4°C.*

PBS++ may be purchased from BioPAL. (Code CL-01-51, Amount 100ml)

*Glutaraldehyde (25%) Store at 4°C.*

25% Glutaraldehyde may be purchased from BioPAL. (Code CL-01-52, Amount 5ml)

*Formalin (40%) Store at 4°C.*

40% Formalin may be purchased from BioPAL. (Code CL-01-53, Amount 5ml)

### Procedure:

**CAUTION: Since cell fixation uses glutaraldehyde and formalin, we strongly advise that all steps using these reagents be performed in a chemical hood. Disposal of these chemicals should follow local, state, and federal regulations.**

1.) **Preliminary steps.** Bring all reagents to room temperature. Warm PBS++ to 37°C.

2.) **Cell fixation solution.** Prepare sufficient cell fixation solution consisting of: 2% formalin and 2.5% glutaraldehyde in PBS++. The recipe for 10ml of cell fixation solution is :

PBS++ 8.5ml

40% formalin 0.5ml

25% glutaraldehyde 1.0ml

3.) Aspirate the medium from the cells.

4.) Wash cells one time with PBS++ warmed to 37°C with a volume equal to the removed medium from Step 3.

5.) Add **cell fixative solution** equal to the volume aspirated from step 3, let it sit for 10 minutes. (The volume of fixative should be equal to that of the volume normally used in the vessel.)

5.) Aspirate the **cell fixative solution** and wash the cells with PBS++ twice.

6.) Add PBS++ to prevent fixed cells from drying.

7.) Stain fixed cells and image as necessary.