

Fixation of Cells with Glutaraldehyde

1. Prepare and wash cells to be fixed in buffered saline. (nutrient medium increases clumping)
2. Suspend pellet very well in 25 times its volume of cold .1% glutaraldehyde in PBS.
3. Let stand 15 min. on ice.
4. Centrifuge
5. Resuspend cells in 0.1M Glycine and incubate for 15 min. in the cold (Glycine binds aldehyde).
6. Centrifuge and wash 3X in distilled water. Store refrigerated.

Glycine MW = 75.07

1 M = 75.07 gm/liter

.1 M = 7.507 gm/liter

pH 6.5

.1% Glutaraldehyde

50% supplied

.5 ml of 50% to 250 ml PBS.

1 ml

50 ml

50 μ

25 ml

25 μ

12.5 ml

Note: the 25% Glutaraldehyde does not polymerize as readily as the 50% - i.e. doesn't become cloudy when mixed w/water