# Preparation for Cell Cycle analysis by Flow Cytometry

## Reagents

- 70% ice-cold ethanol
- Trypsin
- 1X PBS
- Propidium iodide (PI)
- RNase A

## Procedures

1. Harvest cells with trypsin and transfer them into a 15mL tube.

2. Sediment the cells by centrifugation at 1500 rpm for 5 min and discard the supernatant.

3. Resuspend cells in 1mL PBS and transfer the cell suspension to a 1.5 ml Eppendorf, if you have less than 1 X $10^6$ cells.

4. Sediment the cells by centrifugation at 1500 rpm and discard the supernatant.

5. Gently resuspend the pellet in 300$\mu$L of PBS per million of cells – very important for good fixation.

6. Add slowly in drops 700 $\mu$L of 100% –20ºC high grade Ethanol per million of cells while slowly vortexing to prevent clumping.

7. Invert the tube a few times to insure proper mixing and a good fixation of cells.

8. Store at –20ºC until day of analysis.

   *Cells can be stored at this point for several weeks.*

9. Sediment the cells by centrifugation at 5000 rpm and remove all the ethanol.

10. Wash cells 3 times with 1X PBS.

11. Ensure all the ethanol has been removed.

12. Resuspend the pellet in 1 mL of PBS with 2.5 to 10 $\mu$g/mL PI and 0.5 mg/mL RNAase A.

13. Incubate for 30 minutes at 37ºC protected from light.

14. Transfer the cell suspension into a 5 mL FACS tube protected from light.