Procedure

- Wash cells in PBS to remove residual FBS or trypsin
- Cells concentration should be 1- 3 x 10^6 cells/ ml
- Resuspend the pellet in 1 ml of ice-cold 70% ethanol: vortex the pellet and continue to vortex adding slowly drop-by-drop ethanol to the pellet
- Fix cells overnight at 4°C (minimum 2 h)
- Vortex the cells few seconds and centrifuge at 300g x 5'
- Pour off the supernatant
- Gently vortex and add 1ml of PI staining solution
- Vortex carefully and incubate for ~ 30' at room temperature on the dark (gentle rocking will help to accelerate staining process and RNA degradation)
- Analyze the samples within 24 hr in a flow cytometer

Reagents

Propidium Iodide (PI) staining solution:

0.5ml 20x PI stock solution

1000 Kunitz units of RNase A

Up to 10 ml with sample buffer

Mix together just before use

20x PI stock solution:

100mg PI

100ml H2O

Sterilize through 0.22mm filter

Store at 4°C in the dark

Sample Buffer:

1g Glucose

1 liter phosphate-buffered saline (HBSS) w/o Ca++ and Mg++

Sterilize through 0.22mm filter

Store at 4°C