Cell Cycle Analysis after PI Staining

Xiaoji Luo, 01/25/08

1. Cells are treated with drugs in 24-well-plate for a certain time.
2. Remove medium
3. Add trypsin 180-200 ul (according to cell plate), incubated in 37°C
4. When cells are floating, add complete 800 ul PBS to suspend cells
5. Transfer cells to flow cytometry tubes [BD], and *vortex vigorously*
6. Add PBS 2 ml
7. Centrifuge 1200 rpm for 5 min, remove supernatant [wash twice if necessary]
8. Add PBS 200 ul to cells and mixing *(vortex vigorously*)
9. Spray 100% ethanol 800 ul to cell suspension (fast and direct), vortex gently
10. Fix the cells at -20°C for 2 h (or more than 2 h)
11. Centrifuge 1200 rpm for 5 min, remove supernatant
12. Wash cells with PBS 1 ml
13. Centrifuge 1200 rpm for 5 min, remove supernatant
14. Put the tubes on ice bath
15. Add 0.25% triton-X100 0.3 ml (PBS dilution), *vortex vigorously*
16. Keep the tubes on ice bath for 5 min
17. Add 3 ml of PBS
18. Centrifuge 1200 rpm for 5 min, remove supernatant
19. Add PI and RNase stain solution 0.3 ml, vortex
    (BD Biosciences Pharmingen, Cat # 550825: PI/RNase Staining Buffer)
20. Wait for 20 min in room temperature
21. Flow cytometry assay (585 nm)