Cell Cycle Analysis and GFP Expression of Zebrafish Embryos by FACS

Lili Jing*

Department of Cell and Molecular Biology, University of Pennsylvania, Philadelphia, USA
*For correspondence: lilijingcn@gmail.com

[Abstract] Cell cycle analysis in combination with GFP expression has been widely used to study the cell cycle distribution of GFP labeled cells. This protocol is a method to analyze the cell cycle of GFP labeled cells from zebrafish embryos.

Materials and Reagents

1. Phosphate buffered saline (PBS) (Life Technologies, Invitrogen™, catalog number: 14040)
2. Fetal bovine serum (FBS)
3. Ethanol
4. PFA (USB)
5. EtOH
6. Formaldehyde
7. RNaseA
8. Hoechst 33342 solution (Life Technologies, Molecular Probes, catalog number: H3570)
9. 40 μm cell strainer (BD Biosciences, catalog number: 352340)
10. Polystyrene FACS tube (BD Biosciences, Falcon®, catalog number: 352054)
11. 50ml Falcon tube (BD Biosciences, catalog number: 352070)
12. PI solution (see Recipes)
13. PBS + 5% FBS (see Recipes)

Equipment

1. FACS
2. Standard tabletop centrifuges
3. Water bath
4. Polystyrene FACS tube
5. Cell strainer

Procedure

A. Prepare embryos for FACS

1. Thaw FBS in 37 °C bath.
2. Make PBS + 5% FBS.
3. Place embryos in a 5 ml polystyrene FACS tube (50 embryos per analysis).
4. Suck off as much liquid as possible and add 1 ml PBS/FBS.
5. Homogenize embryos at ~1/4 speed maximum for a few sec.
6. Check to see that there are no clumps of embryo/cells.
7. Label 1x 50 ml tubes for each tube of embryos.
8. Put 40 µm cell strainer onto 1 set of 50 ml tubes.
9. Pipet homogenized embryos onto filter.
10. Rinse tube with 1 ml PBS/FBS and pipet onto filter.
11. Transfer filtered embryos into a 5 ml polypropylene FACS tube.
12. Centrifuge at 2,000 rpm, 5 min.
13. Pipet off supernatant.
    If not fixing, resuspend in 500 µl PBS/FBS and leave on ice.

B. Fix cells with formaldehyde and fix/permeabilize with ethanol

1. Resuspend cells gently in 500 µl cold 0.9x PBS.
2. Add 500 µl cold 2% PFA (in 0.9x PBS). Mix gently.
3. Incubate for 30 min, on ice (make sure 70% EtOH in freezer).
4. Centrifuge at 2,000 rpm, 5 min, at 4 °C.
5. Pipet off supernatant (collect in separate PFA waste container) and wash once in 1 ml cold 0.9x PBS.
6. Centrifuge at 2,000 rpm, 5 min, at 4 °C.
7. Pipet off supernatant.
   For Hoechst staining, go directly from PFA fix to Hoechst protocol below.
8. Slowly add 1 ml 70% ethanol at -20 °C drop wise to the cell pellet with the tube sitting on a vortex.
9. Incubate for 30 min, on ice.
10. While incubating, prepare PI solution (In foil).
11. Centrifuge at 2,000 rpm, 5 min, at 4 °C.
12. Resuspend in 500 µl PI solution.
    For cells that are just being fixed, without PI, resuspend in 500 µl 0.9x PBS.
13. Incubate at room temperature ~30 min, in dark.
14. Filter cells on 40 µm filter into 50 ml tube.

15. Transfer cells to 5 ml polystyrene FACS tube.
16. Place on ice then bring to FACS.

C. Hoechst staining

1. Prepare Hoechst 33342 solution—1:1,000 of 1 mg/ml stock in 0.9x PBS.
2. Incubate at 28 °C for ~30 min, in dark.
3. Filter cells on 40 µm filter into 50 ml tube.
4. Transfer cells to 5 ml polystyrene FACS tube.
5. Place on ice then bring to the FACS equipment.

Recipes

1. PI solution (in foil)
   PI + RNaseA in 0.9x PBS
   500 µl 1 mg/ml PI stock (20x)
   100 µl 10 mg/ml RNase A stock (1 µl/ml)
   9.4 ml 0.9x PBS
2. PBS + 5% FBS
   9.5 ml 0.9x PBS + 0.5 ml FBS

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