Cell Cycle Analysis Using Propidium Iodide Staining with GFP Detection

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[Abstract] Infecting mammalian cells with a GFP construct to overexpress or knockdown target genes is one of the most commonly used methods to study and manipulate gene expression. To determine the target gene function on the cell cycle, analyzing the cell cycle (propidium iodide, PI staining) of GFP positive cells vs GFP negative cells is needed. Usually simple fixation of cells with 70% EtOH for PI staining tends to quench GFP signal; paraformaldehyde (PFA) fixation before ETOH fixation could help to sustain the GFP signal.

Materials and Reagents

1. Phosphate buffered saline (PBS)
2. Glucose (Sigma-Aldrich, catalog number: G8270)
3. Paraformaldehyde (Electron Microscopy Sciences, catalog number: 15170)
4. 70% EtOH
5. Hepes (Sigma-Aldrich, catalog number: H3375)
6. NP-40 (MBL International, catalog number: JM-2111-100)
7. BSA (Sigma-Aldrich, catalog number: A3803)
8. RNase A (Sigma-Aldrich, catalog number: R4642)
9. Fix solution (see Recipes)
10. Wash solution (see Recipes)

Equipment

1. Centrifuges (Beckman Falcon, TLS-55)
2. 15 ml polypropylene falcon tubes (BD Biosciences, Falcon®, catalog number: 352097)
3. FACS machine

Procedure

1. Trypsinize and harvest cells, suspend in 10 ml PBS into 15 ml polypropylene falcon tubes.
2. Pellet cells at 1,500 rpm, 3 min, aspirate supernatant.

3. Wash cells 1x in 5 ml PBS.
4. Pellet cells at 1,500 rpm, 3 min, aspirate supernatant.
5. Thoroughly resuspend cells 1 ml fix solution.
6. Incubate 10 min on ice.
7. Add PBS up to 14-15 ml.
8. Pellet cells at 1,500 rpm, 3 min, aspirate supernatant.
9. Wash cells 1x in 5 ml PBS.
10. Resuspend cells in 0.5 ml 1x PBS. Vortex tube gently and add 4.5 ml ice cold 70% EtOH dropwise over 30 sec to 1 min. Incubate on ice > 1 h.
11. Pellet cells at 1,500 rpm, 3 min, aspirate supernatant.
12. Wash cells 1x in wash solution.
13. Pellet cells at 1,500 rpm, 3 min, aspirate supernatant.
14. Resuspend cells in 500 µl of PBS containing 10 µg/ml RNase A and 20 µg/ml PI, transfer to FACS tubes and incubate at room temperature in the dark for 30 min.
15. FACS immediately or store at 4 °C until FACS analysis.

Recipes

1. Fix solution
   1x PBS
   2% Glucose
   3% Paraformaldehyde
2. Wash solution
   1x PBS
   20 mM Hepes
   0.25% NP-40
   0.1% BSA

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References

1. Zhu, H., Coppinger, J. A., Jang, C. Y., Yates, J. R., 3rd and Fang, G. (2008). FAM29A promotes microtubule amplification via recruitment of the NEDD1-gamma-tubulin complex to the mitotic spindle. *J Cell Biol* 183(5): 835-848.

