Cell cycle analysis by flow cytometry in fixed cells

1. Background and purpose of the procedure

When stained with a cell cycle reagent, DNA in the cells bind the dye stoichiometrically (in proportion to the amount of DNA present in each cell). The flow cytometric analysis of cell count versus linear fluorescence is used to create a histogram of the DNA content distribution across the steps of the cell cycle. There are standard modeling algorithms that can then be employed to determine the breakdown of cells in the G0/G1 phase versus S phase, G2, or polyploidy state of the cell population.

2. Materials and equipment

- Adherent cells treated with pharmacological or radiological agent
- D-PBS, without Ca^{++} and Mg^{++} (Gibco, 14190144)
- 0.25% Trypsin, 0.1%EDTA (Corning, 25053CI)
- Medium for cell culture containing 2% FBS
- 70% Ethanol solution at -20˚C
- FACS Buffer (*see 3. Recipes*)
- Hoechst 33342 staining solution (*see 3. Recipes*)

- Flow cytometer equipped with 350 nm laser and able to read emission at 461 nm (DAPI channel)
- Ice bucket
- Ice

3. Recipes

a. FACS Buffer

2% Fetal Bovine Serum (Gibco, 10437028) *[2 mL for 100 mL]*
D-PBS D-PBS, without Ca^{++} and Mg^{++} (Gibco, 14190144) *[98 mL for 100 mL]*

Solution can be stored at 4˚C for up to 3 weeks.

b. Hoechst 33342 stock solution

1 mg/mL Hoechst 33342 (Thermo Fisher Scientific, H1399) *[10 mg for 10 mL]*
DMSO qsp 10 mL (Fisher Chemical, Certified ACS, D128-1)

Aliquot stock solution in 1.5 mL amber microtubes and store at -20˚C until use. Solution can be stored at -20˚C for up to 12 months and is stable for few weeks at 4˚C.

c. Hoechst 33342 staining solution

2 µg/mL Hoechst 33342 *[2 µL of Hoechst 33342 stock solution (b) for 1 mL]*
FACS Buffer (a) qsp 1 mL

Staining solution cannot be stored and must be prepared no more than 15-25 min prior to incubation of cells.

4. Method

a. Gently aspirate the cell culture media from the tissue culture plate or dish
b. Rinse cells using D-PBS without Ca^{++} and Mg^{++}

c. Detach cells with Trypsin-EDTA *[300 µL for 12-well plate, 500 µL for 6-well plate, 1mL for 10cm dish]*
d. Incubate at 37˚C for 1 to 3 min
e. Add cell culture media containing 2% FBS to inhibit Trypsin *[600µL to 3 mL]*
f. Transfer to a centrifuge tube *[1.5 mL or 15 mL depending on total volume]*
g. Pellet cells by centrifugation at 350 g for 5 min at room temperature
h. Resuspend pellet in FACS buffer *[100µL to 500 µL]*
i. Fix and permeabilize cells by adding 70% ethanol at -20˚C in a dropwise manner while gently vortexing *[5 to 8 times FACS buffer volume from (h)]*
j. Incubate cells on ice for 30 min *[cells can be stored at -20˚C in 70% ethanol for few weeks if needed]*
k. Pellet cells by centrifugation at 350 g for 5 min at room temperature
l. Resuspend cells in Hoechst 33342 staining solution *[150µL to 500 µL]*
m. Incubate cells on ice for 30 min
n. Analyze fluorescence by flow cytometry at λ_{ex} 350 nm and λ_{em} 461 nm (DAPI channel)
o. Analyse data using ModFit software

5. References

Darzynkiewicz Z, Huang X, Zhao H. Analysis of Cellular DNA Content by Flow Cytometry. Curr Protoc Cytom. 2017 Oct 2;82:7.5.1-7.5.20. doi: 10.1002/cpcy.28. PMID: 28967991.

The Molecular Probes Handbook, Thermo Fisher Scientific/Invitrogen, Section 15.4 - Assays for Cell Enumeration, Cell Proliferation and Cell Cycle

6. Revision history

Revision #	Date	Prepared by
1.0	**2021-09-24**	**Elie Besserer-Offroy**
Summary of modifications		
Initial version of the protocol		