Measurement of GFP Expression and DNA Content in Fixed Cells

Reagents

Cells to be studied, expressing green fluorescent protein (GFP). Note that the same cell type without GFP is needed as a control for setting up the flow cytometer.

1 X PBS

2% Buffered formaldehyde solution (see recipe)

70% Ethanol

Propidium iodide stock solution (1mg/ml in PBS, PI e.g., Cat# 537059 EMD Millipore, MA)

DNAse-free ribonuclease A (e.g., Cat# R4875 Sigma-Aldrich, St. Louis, MO)

12 X 75 mm culture tubes

Vortex mixer

Water bath at 37^{o}C

Method

Fix cells with formaldehyde

1. Count cells.

2. Place approximately 10^6 cells into a 12 x 15 mm test tube and wash them once with PBS by centrifugation for 5 min at 300 x g at 2-8^{o}C.

3. Remove supernatant by aspiration or rapid decanting and add 0.5 mL of cold PBS to the cell pellet. Mix gently. Add 0.5 mL of cold, buffered 2% formaldehyde solution and mix again. Incubate at 2-8^{o}C for 1h.

Permeabilize cells with ethanol

4. Spin cells down by centrifugation for 5 min at 300 x g at 2-8^{o}C, remove supernatant by aspiration or rapid decanting, wash once with cold 1 X PBS, then add 1 ml of 70% ethanol at –20 ^{o}C drop-wise to the cell pellet with the tube sitting on a vortex. Incubate cell suspension overnight at 2-8^{o}C.

5. Spin cells down by centrifugation for 5 min at 300 x g at 2-8^{o}C, remove supernatant by aspiration or rapid decanting and add 1 ml of a solution containing 40µg/mL of PI and 100 µg/mL of ribonuclease A. Incubate cell suspension at 37^{o}C in the dark for 30 min. If needed, filter samples through a nylon mesh to remove clumps before acquisition on the flow cytometer.

Preparation of Buffered 2% Formaldehyde Solution

Formaldehyde fixative - 2% formaldehyde solution in protein-free PBS.

Prepare as follows:

10% formaldehyde*	20 ml
10 x PBS	10 ml
Distilled water	70 ml

* 10% formaldehyde solution (e.g., Polysciences, Warrington, PA, ultrapure, Cat.#04018), depolymerized paraformaldehyde, EM grade, methanol-free solution.

References

Chu, YW, Wang R., Schmid I, Sakamoto KM. Analysis with flow cytometry of green fluorescent protein expression in leukemic cells. *Cytometry* 36:333-339, 1999.

Schmid I. and Sakamoto KM. Analysis of DNA content and green fluorescent protein expression. *In*: Current Protocols in Cytometry, Vol 1, Robinson JP, Darzynkiewicz Z, Dean P, Orfao A, Rabinovitch P, Stewart C, Tanke H, Wheeless L, eds., John Wiley & Sons, 2001, pp. 7.16.1-7.16.10.