Quantification of Apoptosis and the Cell Cycle Distribution of Primary B Cells Using Propidium Iodide

AfCS Procedure Protocol ID PP0000002400
Version 1, 02/13/02

The water soluble, DNA intercalator, propidium iodide (PI), is used to bind to DNA after permeabilization of cells with NP40. The amount of dye bound correlates with the content of DNA within a given cell. Once cells are stained, they are analyzed on a flow cytometer. The relative content of DNA indicates the distribution of a population of cells throughout the cell cycle. For example, cells in the G_0/G_1 phases of the cell cycle are diploid, or have a DNA content of 2n. Cells within the G_2/M phases have a DNA content of 4n, while S-phase cells have a DNA content greater than 2n and less than 4n. Cells that are sub-diploid (<2n) are apoptotic and can also be quantified using this method.

Procedures

1. Remove 1 x 10^6 cells from the appropriate samples and place into microfuge tubes.
2. Centrifuge in a microfuge at 400 x g for 5 min at 4 °C.
3. Aspirate the supernatants.
4. Cap the tubes. Invert the tubes (approximately 150 degrees) and flick the conical end with a finger 3 to 4 times to loosen the pellets.
5. Add 500 µl of fluorescence-activated cell sorting buffer (FACS buffer) to each sample and gently invert approximately 3 times to wash the cells.
6. Centrifuge in a microfuge for 5 min at 400 x g at 4 °C.
7. Aspirate the supernatants.
8. Add 250 µl of propidium iodide staining solution (PI solution) to each sample.
9. Place the tubes on ice in an ice bucket and cover.
10. Incubate for 30 min.
11. Add 500 µl of FACS buffer to each tube.
12. Transfer each sample from a microfuge tube to a FACS tube.
13. Mix and analyze by passage through a FACSCalibur flow cytometer.

Reagents and Materials

Fluorescence-activated cell sorting buffer (FACS buffer): AfCS Solution Protocol ID PS0000002900

Propidium iodide staining solution (PI solution): AfCS Solution Protocol ID PP0000004400

FACS tubes (Falcon polystyrene round-bottom tubes, 12 x 75 mm): Falcon; catalog no. 35-2054

FACSCalibur flow cytometer: Becton Dickinson; catalog no. 343023

Author: Robert Hsueh/Tamara Roach

Date: 02/15/02

Approved: Paul Sternweis/Bill Seaman