Measurement of Green Fluorescent Protein Expression and DNA Content in Unfixed Cells

Reagents

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

Hoechst 33342 stock solution (1mg/ml) (see recipe using 10mg/mL solution, e.g., Cat# H3570 LifeTechnologies, Grand Island, NY)

12 X 75 mm culture tubes

Vortex mixer

Water bath at 37^{o}C

Method

1. Count cells.

2. Place approximately 10^6 cells into a 12 x 15 mm test tube and spin them down by centrifugation for 5 min at 300 x g.

3. Remove supernatant by aspiration or rapid decanting and add 0.5 mL of the medium that was used for growing the cells to be studied pre-warmed to 37^{o}C to the cell pellet. Mix gently. Add 5 µL of Hoechst 33342 stock solution and mix again. Incubate cells protected from light at 37^{o}C for 45 min.

The optimal Hoechst dye concentration and staining time for different cell types vary as dye up-take depends on cellular metabolic rates; thus, both have to be determined empirically. In general, dye concentrations between 1µg/ml and 10 µg/ml and incubation times between 20 min and 90 min will produce DNA histograms with acceptable coefficients of variation. Because Hoechst DNA staining is performed on unfixed cells, it is possible to use other non-vital DNA dyes, e.g., propidium iodide, 7-aminoactinomycin D, for concurrent dead cell discrimination.

Preparation of Hoechst 33342 stock solution*:*

Dilute 10mg/mL Hoechst 33342 stock solution to 1mg/mL in distilled H2O. Keep solution at 4°C protected from light. Solution can be stored for at least up to 6 months.

Reference

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.