Cell Cycle Analysis using Hoechst 33342 in Unfixed Cells

1. Test principle

Hoechst 33342 is a premeable DNA dye that binds preferentially to A-T base-pairs. Fixation and permeabilization is not required for labeling cells, but physiologic conditions are required, since the dye internalization is accomplished by the ABC (ATP-binding cassette) transporter activity. This condition varies among cell types, so some adjustment may be needed depending on the cell types.
This staining is excellent for simultaneous detection of GFP expression and cell cycle anlaysis.

2. Specimen

Cells to be studied.

3. Materials and reagents

- 5ml Falcon polypropylene Round-Bottom test tubes (12 X 75 mm)
- Vortex mixer
- Cooling centrifuge
- Water bath at 37°C
- **Hoechst 33342 stock solution (1mg/ml)**
  Preparation of Hoechst 33342 stock solution (1mg/ml): Dissolve 1 mg of Hoechst 33342 powder (Molecular Probes, Eugene, OR) in 1 ml of distilled water or DMSO. Store at 2-8°C protected from light for up to 1 month.
- Flow cytometer equipped with a UV laser and a 488 nm laser

4. Controls

1. Untreated cells stained with Hoechst 33342
2. If expressing green fluorescent protein (GFP), note that the same cell type without GFP is needed as control.

5. Procedure

1) Count cells.
2) **Prepare cells:** 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) **Staining cells with Hoechst 33342:** Remove supernatant and add 500 µl of the medium that was used for growing the cells to be studied pre-warmed to 37°C to the cell pellet. Mix gently. Add 5µl of Hoechst 33342 stock solution and mix again. Incubate at 37°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., PI, 7-aminoactinomycin D (7-AAD), for concurrent dead cell discrimination.

4) **Acquire fluorescence data on the flow cyteomter.**

- Hoechst 33342 will also work with parafomraldehyde or ethanol-fixed cells with modification to the above protocol. The reduction of Hoechst 33342 concentration and incubation time (to ~15 minutes) may be needed, since the cells are fixed.

Reference: Current Protocols in Cytometry (2001), 7.16., Ingrid Schmid and Kathleen M. Sakamoto