

**Cell Cycle Analysis using Hoechst 33342**  
(Greg A. Perry, Ph.D.)

**Equipment:**

12x75 mm test tubes

**Reagents:**

Cell preparation (at  $2 \times 10^6$  cells/ml)  
Hoechst staining reagent (0.5 mg/ml)

**Method:**

- 1) Place 0.5 ml of cell preparation ( $10^6$  cells) in 12x75 mm tube.
- 2) Add 2  $\mu$ l of Hoechst staining reagent and mix well. (*Final concentration = 2  $\mu$ g/ml*)
- 3) Incubate 20 minutes at 37°C.
- 4) Analyze on flow cytometer at low flow rate (~150 cells/second or less).

**Notes:**

- Different cell types may require different concentrations of Hoechst (2-5  $\mu$ g/ml final concentration) or different staining times (20-90 minutes). Optimal staining conditions should be determined for each cell source.