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

**Equipment:**

12x75 mm test tubes

**Reagents:**

- Cell preparation (at 2x10^6 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 μl of Hoechst staining reagent and mix well. *(Final concentration = 2 μg/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 μg/ml final concentration) or different staining times (20-90 minutes). Optimal staining conditions should be determined for each cell source.