

## **Hoechst 33342 Cell Staining and Side Population Purification Protocol**

(see Goodell, M., et al. (1996) J Exp Med 183, 1797-806)

The ability to discriminate Hoechst SP cells is based on the differential efflux of Hoechst 33342 by a multi-drug-like transporter. This is an active biological process. Therefore, optimal resolution of the profile is obtained with great attention to staining conditions. The Hoechst concentration, staining time, and staining temperature are all CRITICAL. Likewise, when the staining process is over, the cells should be maintained at 4°C in order to prohibit further dye efflux.

- 1) Ensure that a water bath is precisely at 37°C. The medium needs to be pre-warmed beforehand.
- 2) To thaw cells from the cryovials, put them directly from dry ice into the 37°C water bath. Once the cells are thawed, wash them once by transferring them to a centrifuge tube, adding 9 mls of their respective media, and spinning for 5 minutes at 1200 rpm in order to remove the cryo-preservants.
- 3) Resuspend at  $10^6$  cells per ml in pre-warmed DMEM+5% FBS. Mix well.
- 4) Add Hoechst to a final concentration of 5 µg/ml (a 200x dilution of the stock).
- 5) Mix the cells well, and place in the 37°C water bath for 90 minutes exactly. Make sure the staining tubes are well submerged in the bath water to ensure that the temperature of the cells is maintained at 37°C. Tubes should be mixed several times during incubation.
- 6) After 90 minutes, spin the cells down in the COLD and resuspend in COLD Phosphate Buffered Saline (PBS).
- 7) All further proceedings should be carried out at 4°C to prohibit leakage of the Hoechst dye. Add 2 µg/ml of 7-AAD to the suspended cells (a 100X dilution of the stock provided) and mix about 5 minutes before FACS analysis. This will allow for the discrimination of dead versus live cells as 7-AAD permeates only cells that do not have an intact membrane.