DNA Analysis of Live Cells (Supravital Staining)

The primary application of this method is for cell sorting, where cells selected on the basis of differences in DNA content can be subcultured for the purpose of analyzing their growth characteristics, testing their sensitivity to drugs, cloning, or expanding their number.

- Wash 1×10^6 cells with 1X PBS. Resuspend cell pellet in 1.0 ml of tissue culture medium.

- Add Hoechst 33342 staining solution to obtain a final dye concentration of 2.0 µg/ml. Incubate 20 min/37°C.

- Do not wash cells. Measure cell fluorescence in a flow cytometer (355 nm laser, 440/40 Bandpass filter). Refer to section “How to Set Up a Cell Cycle Experiment (Flow Cytometry)”.

  ✓ **Hoechst 33342 staining soln., 1 mg/ml**: Dissolve 1 mg Hoechst 33342 in 1.0 ml. Store in dark or foil-wrapped bottles several months at 0°C to 4°C.

NOTE: Hoechst 33342 can be replace by DRAQ5: at 488 nm laser, 780/60 Bandpass filter; at 640 nm laser, 660/20 Bandpass filter). Do not recommended in combination with other far-red fluorochromes excited by 488 nm and 633 nm laser.