Hoechst 33342 Staining of Viable Cells for cell cycle analysis/sorting

- For cell cycle analysis and sorting of unfixed samples
- Requires ultraviolet argon laser excitation, 450nm emission
- Binds preferentially to A-T base regions in DNA
- Non-intercalative DNA binding
- DiOC5 can increase resolution of DNA distribution

Reagent list

1. Hoechst 33342 Staining solution (HO)
2. Hoechst 33342 1.0 mg/ml in dH2O
3. DiOC5 (Molecular Probes) 1.0mg/ml in DMSO

Staining

1. HO is added to cells in culture medium at from 1.0-5.0 ug/ml.
2. Cells are incubated in HO at 37 degrees C for 30-60 minutes.
3. Cells are analysed without washing while in the media containing the HO.

Tips

- DO NOT SUBSTITUTE HOECHST 33258 FOR VIABLE CELL STAINING.
- HO stock should not be mixed in PBS, the dye will precipitate.
- Stock dye solution may be frozen and is stable for at least one month in the refrigerator in foil.
- Adherent cells should be stained in vitro, then trypsin and trypsin-neutralizing solutions used should contain the same dye concentration
- Improved resolution (CV'S) can be obtained by adding 0.1-0.3ug/ml DiOC5 at the same time as HO incubation.