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:

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- Hoechst 33342 Staining solution (HO)
        Hoechst 33342   1.0 mg/ml in dH2O
- DiOC5 (Molecular Probes) 1.0mg/ml in DMSO
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PROTOCOL:

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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.
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TIPS:

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