Detection of Apoptosis in unfixed samples using Hoechst 33342

- For the quantitation of apoptosis due to variation in dye uptake
- For unfixed cells
- Can be used simultaneously with cell surface immunofluorescence staining
- Apoptotic cells stain brighter than non-apoptotic after brief exposure to Hoechst 33342
- Requires UV excitation , emission around 450nm

REAGENT LIST:

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- Hoechst 33342 Staining Solution (HO)
        Hoechst 33342   1.0 mg/ml in dH2O
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PROTOCOL:

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Staining:
        1. Wash and resuspend cells at 1-2x10E6 cells/ml of
           preferred cell culture media.
        2. Incubate cells for 10-15 minutes at 37 degrees C
           in a final concentration of 1ug/ml HO 33342
        3. Place cells immediately on ice after incubation to
           minimize further HO uptake.
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TIPS:

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        1. Hoechst 33342 stock should only be mixed in water,
          dye will precipitate out of PBS.
        2. Apoptotic cells are determined by their lower forward
           light scatter, higher side scatter, and intermediate staining inter
        3. If disrimination between apoptotic and viable cells
           is difficult, try shorter incubation periods with dye.
        4. Cells need not be washed out of the HO
        5. Logarithmic amplification of the HO signal is used.
        6. When used with immunofluorescence, complete
           HO staining first, then proceed with immunofluorescence
           labelling on ice.
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REFERENCE:

Hardin, J.A., Sherr, D.H., DeMaria, M., Lopez,P.A. (1992) A simple fluorescence methoc
phenotyping of lymphocytes undergoing DNA fragmentation. J. Immunol. Methods 154, 99.

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