Propidium Iodide Staining for Cell Cycle Determination

- For the quantitation of cell cycle
- For intact fixed cells
- Can be used simultaneously with cell surface or cytoplasmic staining
- Cells stain with a bright red fluorescence (peak emission 610nm)

REAGENT LIST:

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-80% Ethanol (on ice)
-Phosphate Buffered Saline (PBS)
-Propidium Iodide Staining Solution (PI)
        Propidium Iodide        0.5 mg/ml in PBS
-RNase A
        RNase A                 50 units/mg
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PROTOCOL:

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Fixation:
        1. Wash cells into PBS on ice.
        2. Add 1ml of iced cell suspension (2x10E6 cells/ml) to 1ml
           of iced 80% ethanol dropwise while vortexing
        3. Allow to incubate a minimum of 30 minutes on ice.

Staining:
        1. Wash cells once into 1.0 ml PBS.
        2. Add 5ul of stock Propidium Iodide to cell suspension
           (1-2x10E6 cells/ml) plus RNase at 50ug/ml.
        3. Incubate for 30 minute at 37 degrees C in the dark.
        4. Place cells on ice
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COMMON PITFALLS:

1. Ethanol fixation can decrease cell number.
2. Inadequate RNase digestion can cause broadening of peak distributions (higher CV's)
3. Inconsistant cell number and or dye concentration can cause cell cycle peak position to vary between samples.

TIPS:

1. Cells need not be washed out of the PI.
2. PI cell cycle may be done in conjunction with any green fluorescent compounds (FITC,FDG...)

Back to the DFCI Core Flow Cytometry Home Page •