Indo-1 Analysis for Calcium Determination

- Ratiometric method for quantitation of internal cellular calcium levels
- Requires ultraviolet illumination (argon ion laser)
- Fluorescence recorded as the ratio of 405nm to 485nm

REAGENT LIST:

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-Indo-1 Staining Solution:
      Indo-1,AM (Molecular Probes I-1223) 50ug
      Anhydrous DMSO                      50ul

-Calcium ionophore:
      4-Bromo-Calcium ionophore A23187 (Sigma B-7272)
                                  1mg/ml in DMSO
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PROTOCOL:

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1- Spin down 1x10E6 cells per tube (1 tube per condition or
                                    variable being tested)
2- Apirate medium
3- Resuspend in 0.2 ml cell culture medium + Indo-1 (2ug/ml indo-1)
4- Incubate 15-30 minutes at 37 deg C.
5- After incubation, complete volume to 1ml using cell culture media.
6- Keep cells on ice until analysis.
7- Briefly warm individual cell aliquots to 37 deg C a few minutes
   before analysis.
8- Cells are run on the cytometer for 30 seconds to establish a
   baseline calcium level, then removed, stimulated, and replaced
   on the cytometer and followed for typically up to 10 minutes.
9- Calcium ionophore positive control uses 5ul of A-23187 solution
   per 10E6 cells.
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TIPS:

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1- Indo-1,AM staining solutions should only be mixed in small
   aliquots.
2- Use calcium ionophore as a positive control, should give
   maximum dynamic range. If response is poor, check system
   and cell loading.
3- Rinse flow system after ionophore control, using 70% ETOH followed
   by buffer rinse; carryover of ionophore can be a problem.
4- Microscopic evaluation of Indo-1 stained samples using UV
   setup (i.e. DAPI,Hoechst) should show diffuse staining-
   excessive staining in cell compartments indicates overstaining
   (too long/too much)
5- Incomplete loading can cause trouble in a number of cell types-
        - cells with high MDR activity may pump Indo out; try
          blocking this using verapamil or cyclosporin A.
        - use Pluronic (Molecular Probes) when loading the cells
        - esterase activity may not be sufficient in some cells
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