

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

- 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

Protocol

1. Spin down 1×10^6 cells per tube (1 tube per condition or variable being tested)
2. Aspirate 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 10^6 cells.

Tips

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