

CALCIUM FLUX MEASUREMENT USING INDO-1

1. Dissolve the contents of 1 vial (50 µg indo-1) in 50 µl dry DMSO. This makes a 1mM solution. T and B cells can be loaded with 1 µM indo-1; granulocytes or monocytes with 1-3 µM and dendritic cells with 3-6 µM Indo-1. Cell concentration on loading should be $1-10 \times 10^6$ /ml.
2. Incubate cells at 37 °C for 45-60 min in a waterbath or incubator. Wash twice in serum free medium or PBS. Resuspend at 10^6 /ml in PBS or serum free medium of choice. Either keep the cells at 37 °C if using immediately or keep on ice until ready for use; prior warming back to RT or 37 °C should be done as Indo-1 fluorescence is temperature dependent.
3. Immunophenotyping if required should then be carried out on ice.
4. Cells can then be analysed on BD LSR which allows up to six-colour analysis. Thus FITC, PE, TC and APC can be used for immunophenotyping. Indo-1 is only loaded into viable cells thus there is no requirement to exclude dead cells by PI.
5. The filter set-up on the BD LSR for Indo-1 (UV excitation only) is for calcium bound Indo-1 violet FL-5 424/44 nm BF filter and unbound Indo-1 green FL-4 530/30nm BF filter. Calcium flux is measured as a Ratio between calcium bound Indo-1 and unbound or FL-5/FL-4 versus time.
6. Full scale deflection of the calcium flux is measured by the addition of ionomycin 1-10 µg/ml. Contribution of internal stores of calcium can be measured by resuspending cells in calcium-free medium and then adding ionomycin.