INDO-1 TO MEASURE THE CONCENTRATION OF CELL CALCIUM

Reagents and Materials

- Murine or human lymphocytes, cell labeling medium, probenecid (100 mM in water basified with 1M NaOH until dissolution, pH between 9 and 10;
Maximum conservation of 1 month at room temperature),
- Indo-1 pentaacetoxymethyl ester (2 mg / ml in DMSO or 2 mM, storage less than 6 months at -20°C),
- Ionomycin (1 mg/ml in DMSO or 100% ethanol, storage less than 1 year at - 20°C),
- Dimethyl sulfoxide (DMSO) or 10% (v/v) bleach in water, NaCl (0.85% [(w/v)] NaCl) or PBS.

- The labeling medium (toxic PBS) is prepared from PBS without calcium or magnesium, containing HEPES (20mM) and ionomycin (3 µg/ml), nigericin (2 µg/ml), carbonyl cyanide m-chlorophenylhydrazone (CCCP : 10 µM from a 1 mM stock solution in DMSO),
- 2-deoxyglucose (40 mM from a 1 M stock solution in water),
- sodium azide (60 mM from a 3 M stock solution in water).
The maximum conservation is from 1 month to 4°C.

- A water bath (30°C or 37°C)
- A flow cytometer (CMF) with a UV source and a sample thermostating system.

Marking of cells with indo-1

1) Collect the lymphocytes and centrifuge for 5 min at 180 G, at room temperature.
Resuspend the cell pellet in the cell labeling medium at a concentration between 10^6 and 10^7 cells/ml.
Use polypropylene tubes to minimize cell loss.

2) Possibility of adding 100 mM probenecid (4 mM final).

3) Add indo-1 to 2 mg/ml for a final concentration of 2 µg/ml.
Incubate 30 min at 30°C or 37°C.

Immunophenotyping can be carried out prior to the incorporation of indo-1, but it must be checked whether the antigenic labeling does not modify the calcium metabolism: to minimize this effect, the antigenic labeling can be carried out at +4°C, followed by an intermediate incubation at 37°C so that the cells recover their initial metabolism before the incorporation of the probe.

4) Centrifuge the cells for 5 min at 180 G, at room temperature.
Resuspend with a pipette without vortexing in the labeling medium at the desired cell concentration (3 x 10^6 cells/ml).
Store cells at room temperature and protect them from light before analysis.
Do not maintain cells at 37°C as the loss and compartmentalization of indo-1 is accelerated.

5) Use a bandpass filter centered on 395 ± 10 nm and another centered on 500 ± 15 nm or 525 ± 15 nm. Use linear amplification.

6) Check the setting of the device and the cellular incorporation by treating the cells (1×10^5 cells in the labeling buffer) with ionomycin at 1 mg/ml for a final concentration of 1 to 2 µg/ml. An immediate response of 100% of the cells is expected. To avoid contamination by ionomycin which can adhere to the plastic of the fluidic system, rinse well with 6% bleach, then with buffer of labeling medium.

7) To calibrate the fluorescence ratio of indo-1 to the concentration of ionized calcium (Ca^+), resuspend the cells in calcium / EGTA and ionomycin buffers.
Put an aliquot of cells marked with indo-1 at 37°C., 5 to 10 min before analysis.
Use 5 x 10^5 cells per test.
Analyze the cells at 37°C in the incorporation medium.
Use NaCl or PBS as the sheath liquid.

8) Simultaneous analysis of the immunophenotype and Ca^{2+}
The simultaneous use of the cell phenotype thanks to anticmps coupled to FITC and PE with the analysis of indo-1 allows the determination of Ca^{2+} in cell subpopulations.