

PROTOCOL: INTRACELLULAR Ca^{2+} USING INDO-1

- 1.) Preparation of Indo-1: (Molecular Probes, Cat.#I-1223, Indo-1 AM, 50ug aliquots)
 - a.) Dissolve contents of vial in 50ul DMSO (Concentration: 1mM)
 - b.) Dilute 1mM stock 1:10 in Ca/Mg free PBS (50ul stock+450ul PBS) for a 100uM working solution
- 2.) Prepare cell suspension of 1ml aliquots at 1×10^6 cells each in cell loading medium.*
- 3.) Add desired amount of Indo-1 dye to cell suspensions (titer for optimal concentration, typically between 1-10uM); mix thoroughly. Incubate samples at 37°C and 5% CO_2 for 30minutes.
- 4.) Following incubation, remove samples from incubator and centrifuge for 5 minutes at 1500RPM. Decant supernatant.
- 5.) Wash cells once in 1ml of medium.

Optional: If combining surface marker detection with calcium flux assessment, add required amount of directly-conjugated antibodies to cell pellet, vortex gently and incubate at room temp. for 15-20min. Add 1 ml of RPMI, mix, spin, decant and resuspend.
- 6.) Add 0.5-1ml of RPMI to cell suspensions; mix gently. Maintain cells at room temperature until acquired on flow cytometer. Cells can be equilibrated at 37°C for up to 5 minutes prior to flow cytometry acquisition if required.
- 7.) Flow cytometric acquisition:
 - a.) Use dual 488nm (for FS/SS and surface antigen) and 350nm (UV) laser excitation, and fluorescence detection filters for Indo-1 at 395nm (Indo-1+ intracellular Ca^{2+}) and 525nm (Indo-1 without intracellular Ca^{2+}).
 - b.) Using linear amplification, adjust voltage such that detection of Indo fluorescence at 525nm is in the upper half of the graph, and detection of Indo fluorescence at 395nm is at lower half of graph.

c.) Obtain baseline data (about 30 acquisition seconds), pause, add agonist and resume sample collection. Time of collection should be sufficient to allow resolution of Ca^{2+} flux (sample dependent, can be up to 10 minutes).

*For discussion of cell medium requirements, see *Technical Considerations* for this section.

References:

June CH, Ryo A, Rabinovitch PS, Measurement of Intracellular Calcium Ions by Flow Cytometry. In: *Current Protocols in Cytometry*, Robinson JP, Managing Editor, Unit 9.8, 2001.

June, Carl H, Rabinovitch, Peter S: Intracellular Ionized Calcium. *Methods in Cell Biology*, 41:149-173, 1994.