Calcium flux: Indo-1 loading and sample staining procedure for simultaneous measurement of intracellular Ca^{2+}

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Introduction

This protocol was used in the above *Nature Immunology* paper.

Reagents

1. 50 mg vial Indo-1 (Cat # I-1203, Molecular Probes, OR)
2. DMSO (Sigma, St. Louis, MO)
3. RPMI 1640
4. Monoclonal antibodies (mAb), conjugated to suitable fluorochromes
5. Ionomycin (Calbiochem, San Diego, CA)
6. 37°C water bath, centrifuge, vortexer.
7. Agonists to test Ca^{2+} flux, e.g. anti-CD3, anti-IgG, ConA.
8. Serum (for RPMI with 2% serum, if cells require serum).

PREPARATION OF INDO-1:

1. Add 150 ml of DMSO to a 50 mg vial of Indo-1, cover with aluminum foil to protect from light.
2. Vortex well, then warm to 37°C for 5 min.
3. Transfer 150 ml of Indo-1 from vial to 4.85 mls of RPMI (=10mM). Wash out vial very well. If not the entire amount of Indo-1 dissolved in DMSO is used, the remainder can be stored dessicated at –20°C for less than 6 months.
4. Cover the tube of 5 ml of 10 mM Indo-1 with foil.
5. Aliquot the appropriate amount of 10mM Indo-1 to the cell suspension (final conc.=1-5 mM). The optimal concentration is dependent on the cell type.
6. Store excess RPMI-diluted 10 mM Indo-1 at 4°C. In our laboratory, the 10 mM Indo-1 solution has been tested for stability up to 24 hrs.

PREPARATION OF IONOMYCIN:

1. Dissolve 1 mg of ionomycin in 1 ml DMSO.
2. Aliquot 13.5 ml of ionomycin solution into vials for later use and store at -20°C for

less than one year.

3. Dilute one 13.5ml vial of ionomycin with RPMI to a volume of 3 mls (=6 mM).
4. Cover the 3 mls of 6 mM working stock with foil to protect from light.
5. 150 ml of working stock ionomycin is added to 300 ml of Indo-1 loaded cell suspension.

Procedure

1. Incubate cells ($2x10^7$/ml) in RPMI with 1-5 mM Indo-1 (acetoxymethyl ester) at 37°C for 40 min for loading.
2. Incubate aliquots of Indo-1 loaded cells with saturating concentrations of e.g., FITC, PE, PerCP, or Tricolor-conjugated antibodies for 20 min. Incubate at 20° to 25 C unless the antigen is subject to capping, otherwise use 4° to 8 C. **Note:** mAbs must be azide free.

Note: set-up single color stained cells for setting appropriate fluorescence compensation on the instrument.

3. Wash cells twice in RPMI and suspend them at the desired concentration (usually $2x10^6$/ml). Higher cell concentrations ($4x10^6$/ml) are required when the cells of interest represent less than 10% of the total population. Cells can be kept at 20° to 25 C unless the antigen is subject to capping, otherwise use 4° to 8 C.
4. Samples should be analyzed shortly after the cells were prepared.
5. Ionomycin (1-3mM final conc.) is used as a positive control for Indo-1 loading and maximum Ca^{2+} flux.

Critical Steps

Special Note:

Indo-1 requires UV excitation. Make sure that you have an instrument with either an argon laser tuned to UV or a helium-cadmium laser available. Because experiments involving the measurement of calcium flux are performed directly on the flow cytometer, pre experiment consultation is strongly

recommended.

References

June CH, Rabinovitch PS. Intracellular ionized calcium. *Methods Cell Biol.* 1994; **41** :149-74.

June CH, Abe R, Rabinovitch PS. Measurement of intracellular calcium ions by flow cytometry. In: *Current Protocols in Cytometry*, **Vol 2**, Robinson JP, Darzynkiewicz Z, Hyun W, Orfao A, Rabinovitch P, eds., John Wiley & Sons, 1997, pp. 9.8.1 – 9.8.19.

Scaffold protein Dlgh1 coordinates alternative p38 kinase activation, directing T cell receptor signals toward NFAT but not NFkappa-B transcription factors

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