

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

June Round

UCLA

Method Article

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Abstract

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 Ca²⁺ 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 desiccated 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 (2x10⁷/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 2x10⁶/ml). Higher cell concentrations (4x10⁶/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 Ca²⁺ 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

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