

T Cell Calcium Mobilization Study (Flow Cytometry)

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[Abstract] Antigen recognition and activation of T cell receptor (TCR) triggers transient calcium release from intracellular compartments and subsequent sustained calcium influx through cell surface *Icrac* channels. Sustained elevation of the cytoplasmic calcium level activates many calcium-dependent enzymes and transcription factors, which are essential for T cell activation and function. This protocol uses non-ratiometric dyes, in combination with flow cytometry, to monitor TCR-triggered calcium changes over time, and is a simple assay to examine the existence of T cell calcium mobilization defects in transgenic mice.

Materials and Reagents

1. Anti-CD3 Armenian hamster primary antibody (BD Biosciences, catalog number: 553057, NA/LE)
2. Goat anti-Armernian Hamster IgG antibody (Jackson ImmunoResearch, catalog number: 127-005-099)
3. Phosphate buffered saline (PBS)
4. CaCl₂
5. MgCl₂
6. DMSO (Merck KGaA, Calbiochem®, catalog number: 540025)
7. Fluo-3 AM in DMSO (Life Technologies, Molecular Probes®, catalog number: F-1242)
(*Note: Fluo-3 fluorescence is calcium-dependent*) (see Recipes)
8. Fura Red AM in DMSO (Life Technologies, Molecular Probes®, catalog number: F-3020)
(*Note: Fura Red fluorescence is calcium-independent. Fura Red serves as a control of dye loading efficiency*) (see Recipes)
9. Pluronic F-127 in DMSO (Life Technologies, Molecular Probes®, catalog number: F-1242)
(see Recipes)
10. Hanks buffered solution (HBSS) (Life Technologies, Gibco®, catalog number: 14170-112)
(see Recipes)
11. Dye loading buffer (see Recipes)

Equipment

1. Flow Cytometry

Procedure

1. Prepare dye loading buffer 2 ml for one sample.
2. Suspend cells at 5×10^6 cells/ml in 1 ml dye loading buffer and incubate 30 min at 37 °C.
3. Spin down cells 5 min at 1,000 rpm.
4. Stain cells with 5 µg/ml anti-CD3 hamster primary antibody (no azide) for 30 min on ice or at 4 °C (0.5 µg/100 µl PBS).
5. Wash cells once.
6. Resuspend cells in 3 ml HBSS/Ca/Mg/FBS at 3×10^6 cells/ml and store at RT and protect from light.
7. For calcium mobilization, warm up samples at 37 °C for 5-10 min. Submit samples to flow cytometry for calcium baseline measurement. After 5 min, add 5 µg/ml goat anti-hamster IgG antibody (15 µg/3 ml), mix well and immediately continue the measurement with flow cytometry. To maintain the incubation temperature, a small beaker containing water prewarmed to 37 °C is necessary to bath sample tubes during the time course of measurement.

Recipes

1. Dye loading buffer (2 ml for one sample)
 - a. HBSS/Ca/Mg/FBS

HBSS	20 ml
1 mM CaCl ₂	26 µl
1 mM MgCl ₂	22 µl
FBS	200 µl
 - b. Dye loading buffer

HBSS/Ca/Mg/FBS	2 ml
10% pluronic F-127	4 µl
4 mg/ml Fluo-3 AM	2 µl
10 mg/ml Fura Red AM	2 µl
2. Hanks buffered solution (HBSS) supplemented with 1.3 mM CaCl₂ and 1.1 mM MgCl₂.
3. 10% (w/v) pluronic F-127 in DMSO, 10%, store at RT.
4. 4 mg/ml Fluo-3 AM in DMSO (20 x 50 µg) aliquot and store in a -20 °C dessicator.

5. 10 mg/ml Fura Red AM in DMSO (500 µg) aliquot and store in a -20 °C dessicator.

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References

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