T Cell Calcium Mobilization Study (Flow Cytometry)

Guo N. Huang*

Program in Biochemistry, Cellular and Molecular Biology, Johns Hopkins University School of Medicine, Baltimore, USA
*For correspondence: guo.n.huang@gmail.com

[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 Armernian 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. $CaCl_2$
5. $MgCl_2$
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 x 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 x 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 $CaCl_2$ and 1.1 mM $MgCl_2$.
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.

Acknowledgments

This work was supported by National Institute on Drug Abuse (NIDA; DA00266, DA10309) and the National Institute of Mental Health (NIMH; MH068830). This protocol was previously used in and adapted from Huang *et al.* (2008).

References

1. Huang, G. N., Huso, D. L., Bouyain, S., Tu, J., McCorkell, K. A., May, M. J., Zhu, Y., Lutz, M., Collins, S., Dehoff, M., Kang, S., Whartenby, K., Powell, J., Leahy, D. and Worley, P. F. (2008). NFAT binding and regulation of T cell activation by the cytoplasmic scaffolding Homer protein. *Science* 319(5862): 476-481.

