Calcium Imaging

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[Abstract] This is a protocol that describes a Ca^{2+} imaging experiment using Ca^{2+} indicator Fura-2. Ca^{2+} imaging is an efficient and quantitative method for measuring cytosolic and internal store Ca^{2+} levels, as well as their dynamic changes.

Materials and Reagents

1. HEK293 cells
2. Phosphate buffered saline (PBS)
3. Fura-2-AM (Life Technologies, Invitrogen™, catalog number: F1221)
4. Poly-L-lysine (Sigma-Aldrich, catalog number: P8920)
5. Fura-2 calcium imaging calibration kit (Life Technologies, Invitrogen™, catalog number: F6774)
6. NaCl
7. $MgCl_2$
8. KCl
9. Glucose
10. HEPES
11. $CaCl_2$
12. DMSO
13. GFP reporter
14. Lonomycin
15. Ca^{2+} recording buffer (see Recipes)

Equipment

1. Zeiss inverted microscopy with perfusion system and “IPlab” software
2. Centrifuges
3. Autoclaved coverslips or multi-well coverglass chambers

Procedure

Note: Target cells: Concentration of Fura-2-AM may need to be optimized depending on cell types to be measured. The following protocols are designed for HEK293 cells.

A. Prepare the cells

1. Autoclaved coverslips or multi-well coverglass chambers are used for culturing cells. Coat the coverslips with 0.01% poly-L-lysine (dilute the 0.1% stock as 1:10 with sterilized water) at RT for 10 min and washed three times with sterilized PBS.
2. Plate HEK293 cells on coverslips 24 h before Ca^{2+} imaging. If cells need to be transfected with GFP reporter, seed cells 48 h before Ca^{2+} imaging and transfect cells 24 h after seeding. For coverslips in 6-well plates, 0.2-0.5 millions of cells should be seeded to each well. Numbers of the cells to be seeded can be adjusted according to the size of plate/chamber used.

B. Load the cells

1. Wash cells with Ca^{2+} recording buffer twice at RT, 5 min each.
2. Dissolve the cell-permeant acetoxymethyl ester of the calcium indicator Fura-2 (Fura-2-AM, 50 μg/vial) in 50 μl of DMSO and then dilute into Ca^{2+} recording buffer.
3. Load HEK293 cells with 1 μg/ml Fura-2-AM at RT for 30 min (Fura-2-AM can be used for up to 5 μg/ml for different types of cells). Wash cells twice with recording buffer, 5 min each time.

C. Prepare perfusion system (Smartsquirt small volume delivery system)

1. Turn on the nitrogen tank with pressure up to 10 psi-20 psi.
2. Turn on vacuum pump.
3. Wash the buffer reservoir.
4. Adjust the pressure regulator to reach a 2 ml/min flow speed.
5. Load the buffer reservoir with Ca^{2+} recording buffer.

D. Load coverslips onto microscopy

1. Place the coverslip in a perfusion chamber and load with 0.5 ml of Ca^{2+} recording buffer.
2. Turn on the Zeiss inverted microscopy; mount the perfusion chamber on the stage of microscopy; use 40x oil emersion lens;
3. Turn on the power of Zeiss FluorArc mercury lamp, and turn the filter to “Fura-2” which will collect the emission response at 510 nm;

E. IPlab software

1. Turn on the camera connecting to microscopy;
2. In IPlab, click the ratiometric icon, set the parameters:

3. Total time points; Interval time (typically 1-10 sec); Exposure time for 340 nm (typically 100 ms); Exposure time for 340 nm (typically 20 ms). Parameters can be adjusted according to experiment design and cells to be measured.
4. Click the “W1” icon to expose cells to 340 nm; adjust the focus plane to get the best image; adjust the exposure time to control the intensity to around 1,000 (the maxi value is 4,999).
5. Click the “W2” icon to expose cells to 380 nm; adjust the exposure time to control the intensity to around 1,000 (the maxi value is 4,999).
6. Select the individual cells to be measured as well as the background region. Multiple cells can be selected in each field.
7. Start recording, image will be acquired by exciting the cells at 340 nm and 380 nm every 3 sec. The ratios of 340/380 nm indicate the intracellular Ca^{2+} concentration.

F. Export data to excel

In IPlab, open the ratio-plot file, from “view” - “new” - “text”, export data as excel files.

G. Applications

1. Measure ER Ca^{2+} content

   For the experiments measuring ER Ca^{2+} content in HEK293 cells, cells were switched from 2 mM Ca^{2+} to nominally Ca^{2+} free (0 mM Ca^{2+}) recording buffer by using the perfusion system, and 2 μM of ionomycin was added to deplete Ca^{2+} stores (Feng *et al*., 2010).
2. Measure Ca^{2+} influx and efflux

   For the experiments measuring Ca^{2+} influx and efflux, incubate HEK293 cells in 0 Ca recording buffer during Fura-2 loading. Measure basal Ca^{2+} level for 10-20 time points to make sure Ca^{2+} level has become stable. Carefully remove recording buffer from the perfusion chamber and rapidly add back recording buffer with different Ca^{2+} concentrations (0.5 mM, 1 mM, 2 mM *et al*.). Keep recording intracellular Ca^{2+} concentration until it reaches the plateau (typicall 3-5 min) (the increase of intracellular Ca^{2+} concentration indicates Ca^{2+} influx). Carefully remove recording buffer from the perfusion chamber and rapidly add back 0 Ca recording buffer. Keep recording intracellular Ca^{2+} concentration until it reaches the plateau (typicall 3-5 min) (the decrease of intracellular Ca^{2+} concentration indicates Ca^{2+} eflux) (Feng *et al*., 2010).
3. Calculation of Ca^{2+} concentration

   Ca^{2+} concentration can be calculated by:

   $[Ca^{2+}] = Kd * (R – R_{min}) / (R_{max} – R) * F_{380max} / F_{380min}$ (Grynkiewicz *et al*., 1985)

R is the measured 340/380 nm ratio; R_{min} and R_{max} are the ratios in absence of Ca^{2+} or when Fura-2 is saturated by Ca^{2+}, and can be determined by incubating cells in 0 Ca recording buffer with 2 mM EDTA or treating cells with 10 μM Ionomycin in recording buffer containing 10 mM Ca^{2+}. F_{380max} and F_{380min} are the fluorescence intensity of 380 nm excitation at 0 Ca and Ca saturation. Kd can be calibrated using the calibration kit from Invitrogen (F-6774).

Recipes

1. Calcium recording buffer
   125 mM NaCl
   2 mM $MgCl_2$
   4.5 mM KCl
   10 mM Glucose
   20 mM HEPES pH 7.4
   2 mM $CaCl_2$ (no $CaCl_2$ was added for the 0 Ca^{2+} buffer)

References

1. Feng, M., Grice, D. M., Faddy, H. M., Nguyen, N., Leitch, S., Wang, Y., Muend, S., Kenny, P. A., Sukumar, S., Roberts-Thomson, S. J., Monteith, G. R. and Rao, R. (2010). Store-independent activation of orai1 by spca2 in mammary tumors. *Cell* 143(1): 84-98.
2. Grynkiewicz, G., Poenie, M. and Tsien, R. Y. (1985). A new generation of Ca2+ indicators with greatly improved fluorescence properties. *J Biol Chem* 260(6): 3440-3450.

