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FM1-43 dye uptake (loading/unloading assays) from Yao CK et al. (2017)

Chi-Kuang Yao, Yu-Tzu Liu, I-Chi Lee, You-Tung Wang, Ping-Yen Wu

Abstract

This protocol is from 'Flower Ca^{2+} channel in CME and ADBE' of Yao CK et al.

Please see the manuscript for the full method details.

Before start

You'll need:

0 mM Ca^{2+} hemolymph-like (HL)-3 solution:

- 70 mM NaCl
- 5 mM KCl
- 10 mM $MgCl_2$
- 10 mM $NaHCO_3$
- 5 mM trehalose
- 5 mM HEPES (pH 7.2)
- 115 mM sucrose

90 mM K^+/0.5 mM Ca^{2+} stimulation:

(or alternative 60mM K^+/1mM Ca^{2+})

- 25 mM NaCl
- 90 mM KCl
- 10 mM $MgCl_2$
- 10 mM $NaHCO_3$

- 5 mM trehalose
- 5 mM HEPES (pH 7.2)
- 30 mM sucrose
- 0.5 mM $CaCl_2$

solution of 90 mM K^+/2 mM Ca^{2+}/200 mM chlorpromazine:

- 25 mM NaCl
- 90 mM KCl
- 10 mM $MgCl_2$
- 10 mM $NaHCO_3$
- 5 mM trehalose
- 5 mM HEPES (pH 7.2)
- 30 mM sucrose
- 2 mM $CaCl_2$
- 200 mM chlorpromazine

Protocol

FM1-43 dye loading/unloading assays
Step 1.

Dissect larvae in 0 mM Ca^{2+} HL-3 solution at room temperature.

FM1-43 dye loading/unloading assays
Step 2.

Subject to a stimulation of 90 mM K^+/0.5 (or 2) mM Ca^{2+} HL-3 solution for 5 min to load boutons with the FM1-43 dye.

⏱ DURATION
00:05:00

FM1-43 dye loading/unloading assays
Step 3.

Remove excess dye by extensive washing with 0 mM Ca^{2+} HL-3 solution for 10 min.

⏱ DURATION
00:10:00

FM1-43 dye loading/unloading assays
Step 4.

Image FM1-43 dye uptake by boutons to indicate "Loading".

FM1-43 dye loading/unloading assays

Step 5.

Subsequently, unload the dye loaded in SVs by stimulation using 90 mM K^+/0.5 (or 2) mM Ca^{2+} solution for 1 min.

⏲ DURATION

00:01:00

FM1-43 dye loading/unloading assays

Step 6.

Remove released dye by several washes with a 0 mM Ca^{2+} HL-3 solution.

FM1-43 dye loading/unloading assays

Step 7.

Image the remaining dye in boutons to indicate "Unloading".

FM1-43 dye loading/unloading assays

Step 8.

Calculate final FM1-43 dye intensity in boutons by subtracting the dye fluorescence intensity in surrounding muscles from the dye fluorescence intensity within boutons.

➕ NOTES

Chi-Kuang Yao 04 Apr 2017

The dye fluorescence intensity of at least 10 type Ib boutons from the same muscles 6 and 7 was averaged to obtain each data value. The dye unloading efficiency was indicated as $(F_{load}-F_{unload})/F_{load}$. Images processing was achieved using Image J and LSM Zen.