FM1-43 dye uptake (CME induction) 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.

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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

Materials

- 70 mM NaCl by Contributed by users
- 5 mM KCl by Contributed by users
- 10 mM MgCl2 by Contributed by users
- 10 mM NaHCO3 by Contributed by users
- 5 mM trehalose by Contributed by users
- 5 mM HEPES (pH 7.2) by Contributed by users
- 115 mM sucrose by Contributed by users
- 25 mM NaCl by Contributed by users
- 90 mM KCl by Contributed by users
- 10 mM MgCl2 by Contributed by users
- 10 mM NaHCO3 by Contributed by users
- 30 mM sucrose by Contributed by users
- 0.5 mM CaCl2 by Contributed by users
- 4 µM fixable FM1-43 (Invitrogen) by Contributed by users
- 4% paraformaldehyde/1XPBS solution by Contributed by users
- 1XPBS by Contributed by users
- glycerol-containing mounting medium by Contributed by users

  200 µM chlorpromazine by Sigma
- Schneider medium by Contributed by users

Protocol

Step 1.

To induce CME, dissect the third instar larvae in 0 mM Ca^{2+} hemolymph-like (HL)-3 solution at room temperature (70 mM NaCl, 5 mM KCl, 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 5 mM trehalose, 5 mM HEPES (pH 7.2) and 115 mM sucrose) [60]

Step 2.

Subject to 1-min 90 mM K^+/0.5 mM Ca^{2+} stimulation (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 and 0.5 mM $CaCl_2$) or 10-min 60 mM K^+/1 mM Ca^{2+} stimulation (55 mM NaCl, 60 mM KCl, 10 mM $MgCl_2$, 10 mM $NaHCO_3$, 5 mM trehalose, 5 mM HEPES (pH 7.2), 30 mM sucrose and 1 mM $CaCl_2$) in the presence of 4 µM fixable FM1-43 (Invitrogen).

Step 3.

Extensively wash out excess dye with 0 mM Ca2+ HL-3 solution for 10 min.

DURATION
00:10:00

Step 4.

Fix larval fillets in 4% paraformaldehyde/1XPBS solution for 10 min.

DURATION
00:10:00

Step 5.

Wash out the fixative by rinsing with 1XPBS solution. (1/3)

Step 6.

Wash out the fixative by rinsing with 1XPBS solution. (2/3)

Step 7.

Wash out the fixative by rinsing with 1XPBS solution. (3/3)

Step 8.

Mount the samples with glycerol-containing mounting medium and image on a Zeiss 780 confocal microscope.

NOTES
Chi-Kuang Yao 29 Mar 2017

The scan setup was fixed for all sets of experiments.

For data quantifications, single-plane confocal images were projected, and final FM1-43 dye intensity in boutons was calculated by subtracting the dye fluorescence intensity in surrounding muscles from the dye fluorescence intensity within boutons.

The dye fluorescence intensities of all type Ib boutons from the same muscles 6 and 7 were averaged to obtain each data value.