

# FM1-43 dye uptake (chlorpromazine treatment ) 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  $\text{Ca}^{2+}$  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 $\text{Ca}^{2+}$ hemolymph-like (HL)-3 solution:

- 70 mM NaCl
- 5 mM KCl
- 10 mM  $\text{MgCl}_2$
- 10 mM  $\text{NaHCO}_3$
- 5 mM trehalose
- 5 mM HEPES (pH 7.2)
- 115 mM sucrose

### 90 mM $\text{K}^+$ /0.5 mM $\text{Ca}^{2+}$ stimulation:

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

- 25 mM NaCl
- 90 mM KCl
- 10 mM  $\text{MgCl}_2$
- 10 mM  $\text{NaHCO}_3$

- 5 mM trehalose
- 5 mM HEPES (pH 7.2)
- 30 mM sucrose
- 0.5 mM  $\text{CaCl}_2$

**solution of 90 mM  $\text{K}^+$ /2 mM  $\text{Ca}^{2+}$ /200 mM chlorpromazine:**

- 25 mM NaCl
- 90 mM KCl
- 10 mM  $\text{MgCl}_2$
- 10 mM  $\text{NaHCO}_3$
- 5 mM trehalose
- 5 mM HEPES (pH 7.2)
- 30 mM sucrose
- 2 mM  $\text{CaCl}_2$
- 200 mM chlorpromazine

## Protocol

### Chlorpromazine treatment experiment

#### Step 1.

Incubate dissected larvae with 200 mM chlorpromazine (Sigma) in Schneider medium for 30 min at room temperature.

 DURATION

00:30:00

### Chlorpromazine treatment experiment

#### Step 2.

Rinse the samples with 0 mM  $\text{Ca}^{2+}$  HL-3 solution. (1/3)

### Chlorpromazine treatment experiment

#### Step 3.

Rinse the samples with 0 mM  $\text{Ca}^{2+}$  HL-3 solution. (2/3)

### Chlorpromazine treatment experiment

#### Step 4.

Rinse the samples with 0 mM  $\text{Ca}^{2+}$  HL-3 solution. (3/3)

### Chlorpromazine treatment experiment

#### Step 5.

Stimulate the samples with a solution of 90 mM  $\text{K}^+$ /2 mM  $\text{Ca}^{2+}$ /200 mM chlorpromazine (25 mM NaCl,

90 mM KCl, 10 mM MgCl<sub>2</sub>, 10 mM NaHCO<sub>3</sub>, 5 mM trehalose, 5 mM HEPES (pH 7.2), 30 mM sucrose, 2 mM CaCl<sub>2</sub> and 200 mM chlorpromazine) in the presence of 4 μM fixable FM1-43 for 10 min.

#### DURATION

00:10:00

Chlorpromazine treatment experiment

#### **Step 6.**

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

#### DURATION

00:10:00

Chlorpromazine treatment experiment

#### **Step 7.**

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

Chlorpromazine treatment experiment

#### **Step 8.**

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

Chlorpromazine treatment experiment

#### **Step 9.**

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

Chlorpromazine treatment experiment

#### **Step 10.**

Mount the samples with glycerol-containing mounting medium.

#### NOTES

**Chi-Kuang Yao** 04 Apr 2017

Bulk membranous invaginations were defined as the internalized structures labeled with high levels of FM1-43 dye. The area of individual type Ib boutons and bulk membranous invaginations were counted using Image J.