Ca2+ imaging (salivary glands) from Yao CK et al. (2017)

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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

2 mM Ca^{2+}/5 mM K^+/7 mM glutamate 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
- 2 mM $CaCl_2$
- 7 mM Monosodium glutamate

4 µM Fluo-4 AM (Invitrogen)/100 mM Ca^{2+}/HL-3 solution to be loaded with Fluo-4 AM dye

Protocol

Ca2+ imaging in salivary glands

Step 1.

Dissect the gland cells of the third instar larvae in 0 mM Ca^{2+} HL-3 solution and plate on poly-L-Lysine-coating coverslip.

Ca2+ imaging in salivary glands

Step 2.

Bath the cells in 4 µM Fluo-4 AM (Invitrogen)/100 mM Ca^{2+}/HL-3 solution to be loaded with Fluo-4 AM dye.

Ca2+ imaging in salivary glands

Step 3.

Capture the images every 10 min during dye loading using MetaMorph software and ANDOR iXon 897 camera.

La3+ treatment

Step 4.

Incubate the cells in 4 µM Fluo-4 AM (Invitrogen)/100 mM Ca^{2+}/100 mM $LaCl_3$/HL-3 solution.

La3+ treatment

Step 5.

Capture the images every 10 min during dye loading using MetaMorph software and ANDOR iXon 897 camera.

Counting

Step 6.

Count the fluorescence intensity in the salivary gland cells and surrounding cover slips.

Counting

Step 7.

Calculate final fluorescence value by subtracting fluorescence intensity in the gland cells from the dye fluorescence intensity in the coverslips.

NOTES

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The fluorescence intensity of one salivary gland was used for each data value. Image processing was achieved using Image J and LSM Zen.