Immunohistochemistry (Immunostaining in fly NMJ boutons) from Yao CK et al. (2017)

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Abstract

This protocol describes the Immunostaining in fly NMJ boutons. It 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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Guidelines

Primary antibody dilutions used: mouse anti-Dlg (mAb 4F3)[57], 1:100 (Hybridoma bank)[58]; mouse anti-Brp (nc82), 1:100 (Hybridoma bank)[59]; rabbit anti-GFP, 1:500 (Invitrogen); mouse monoclonal anti-HA, 1:200 (Sigma); rabbit anti-HA, 1:200 (Sigma); rabbit Cy3 conjugated anti-HRP, 1:500 (Jackson ImmunoResearch); guinea pig anti-Fwe-PB (GP100Y), 1:100. Secondary antibodies were diluted in 1:500 (Jackson ImmunoResearch and Invitrogen).

To compare the staining intensity of boutons among different genotypes, larval fillets used in the same graph were stained in the same eppendorf. The images were captured using a Zeiss 780 confocal microscope, and the scan setup was fixed for the same experimental set. For data quantifications, single-plane confocal images were projected. The final staining intensity in boutons was calculated by subtracting background fluorescence intensity in the surrounding muscles from the staining intensity in boutons. The staining intensity of all type Ib boutons from the same muscles 6 and 7 in one image was averaged to obtain each data value. The dye unloading efficiency was calculated from F_{load}-F_{unload})/F_{load}. Images processing was achieved using LSM Zen and Image J.

Materials

- 4% paraformaldehyde/1XPBS solution by Contributed by users
- 1XPBS solution by Contributed by users
- 0.1% Triton X-100-containing 1XPBS solution by Contributed by users
- 0.1% Triton X-100-containing 1XPBS solution/5% normal goat serum by Contributed by users
- glycerol-containing mounting medium by Contributed by users

Protocol

Immunostaining in fly NMJ boutons

Step 1.

Dissect larval fillets in ice-cold 1XPBS solution for immunostaining in fly NMJ boutons.

Immunostaining in fly NMJ boutons

Step 2.

Fix larval fillets in 4% paraformaldehyde/1XPBS solution for 20 min at room temperature.

DURATION
00:20:00

Immunostaining in fly NMJ boutons

Step 3.

Collect the samples in 0.5 ml eppendorf in 1XPBS solution.

Immunostaining in fly NMJ boutons

Step 4.

Permeabilize the samples in 0.1% Triton X-100-containing 1XPBS solution for 20 min at room temperature on a rotator. (1/3)

DURATION
00:20:00

Immunostaining in fly NMJ boutons

Step 5.

Permeabilize the samples in 0.1% Triton X-100-containing 1XPBS solution for 20 min at room temperature on a rotator. (2/3)

DURATION
00:20:00

Immunostaining in fly NMJ boutons

Step 6.

Permeabilize the samples in 0.1% Triton X-100-containing 1XPBS solution for 20 min at room temperature on a rotator. (3/3)

DURATION
00:20:00

Immunostaining in fly NMJ boutons

Step 7.

Incubate the samples with the staining solution at least for 8 hr at 4° C on a rotator.

DURATION
08:00:00

NOTES
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For staining with anti-Fwe (GP100Y) and anti-HA antibodies, use 0.1% Tween-20-containing 1XPBS solution to prevent Fwe dissociation from SVs. Dilute Primary antibodies in 0.1% Triton X-100-containing 1XPBS solution/5% normal goat serum.

Immunostaining in fly NMJ boutons

Step 8.

Wash the sample with 0.1% Triton X-100-containing 1XPBS solution for 30 min at room temperature on a rotator. (1/3)

DURATION
00:30:00

Immunostaining in fly NMJ boutons

Step 9.

Wash the sample with 0.1% Triton X-100-containing 1XPBS solution for 30 min at room temperature on a rotator. (2/3)

DURATION
00:30:00

Immunostaining in fly NMJ boutons

Step 10.

Wash the sample with 0.1% Triton X-100-containing 1XPBS solution for 30 min at room temperature on a rotator. (3/3)

DURATION
00:03:00

Immunostaining in fly NMJ boutons

Step 11.

Dilute secondary antibodies in 0.1% Triton X-100-containing 1XPBS solution/5% normal goat serum.

Immunostaining in fly NMJ boutons

Step 12.

Incubate the samples with this staining solution at least for 2 hr at room temperature on a rotator.

DURATION
02:00:00

Immunostaining in fly NMJ boutons

Step 13.

Wash the sample with 0.1% Triton X-100-containing 1XPBS solution for 30 min at room temperature on a rotator. (1/3)

DURATION
00:30:00

Immunostaining in fly NMJ boutons

Step 14.

Wash the sample with 0.1% Triton X-100-containing 1XPBS solution for 30 min at room temperature on a rotator. (2/3)

DURATION
00:30:00

Immunostaining in fly NMJ boutons

Step 15.

Wash the sample with 0.1% Triton X-100-containing 1XPBS solution for 30 min at room temperature on a rotator. (3/3)

DURATION
00:30:00

Immunostaining in fly NMJ boutons

Step 16.

Replace the wash solution with glycerol-containing mounting medium.

Immunostaining in fly NMJ boutons

Step 17.

Mount the samples further with glycerol-containing mounting medium.