Flourescent Immunostaining Protocol for a-Bungorotoxin (AChRs) in Zebrafish

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[Abstract] Zebrafish embryo is convenient for studying the neuromuscular system due to its transparency. The protocol described here is a rapid method to visualize AChR clusters on the post-synaptic muscle membrane.

Materials and Reagents

1. Paraformaldehyde (USB Corporation, catalog number: 19943)
2. Phosphate buffered saline (PBS)
3. DMSO
4. Triton-100
5. Na_2HPO_4
6. NaH_2PO_4
7. Collagenase (Sigma-Aldrich, catalog number: C-9891)
8. α-Bungorotoxin conjugated to Alexa 594 (Life Technologies, Invitrogen™, catalog number: B-13423)
9. Vectashied (Vector Lab, catalog number: H-1400)
10. Primary antibody
11. Secondary antibody
12. Incubation Buffer (IB) (see Recipes)
13. 0.1 M phosphate buffer(7.4) (see Recipes)

Equipment

1. Rotator (Storvall Life Science, the Belly Dancer)
2. Fluorescence microscope
3. Transfer pipette

Procedure

1. Dechorinate embryos and remove all E3 buffer.

2. Fix the embryos using 4% paraformaldehyde in PBS+1% DMSO at RT for 4 h or O/N at 4 °C.
3. Remove paraformaldehyde with a transfer pipette. Wash 3 x 10 min with PBS (don't use PBST).
4. Add 0.1% (1 mg/ml in 1x PBS) collagenase and incubate at RT. Treatment times vary according to age: up to 24 h=6 min; 24 to 36 h=9 min; 48 h=45 min; 3 d=75 min; 4 d=?; 5 d=Don't collagenase treat, peel larvae using sharp forceps instead.
5. Remove collagenase solution. Wash 3 x 5 min in PBS.
6. Incubate 30 min in 10 µg/ml a-Bungorotoxin conjugated to Alexa 594 (molecular probe B-13423) in IB at RT.
7. Wash 3 x 5 min in IB.
8. If doing double staining, incubate the embryos in primary antibody diluted in IB O/N at 4 °C. Otherwise go to step 11.
9. Remove primary antibody, wash 6 x 10min in IB.
10. Remove IB, replace with secondary antibody diluted in IB.
11. Remove secondary antibody, wash 6 x 10 min in IB.
12. Remove IB, replace with 1 drop Vectashield. Let embryos settle to bottom of tube overnight. Store embryos at 4 °C.
13. Mount embryos in Vectashied.

Recipes

1. Incubation Buffer (IB) (400 ml)

BSA	0.8 g
10%Triton-100	20 ml
0.1M phosphate buffer (7.4)	380 ml
Store at 4 °C	

2. 0.1 M phosphate buffer(7.4) 400 ml

0.2 M Na_2HPO_4	162 ml
0.2 M NaH_2PO_4	38 ml
H_2O	200 ml

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