

## MEI Protocol

### Whole-mount immunochemistry

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Dissected muscles were fixed in 4% PFA at 4°C overnight, rinsed with PBS (pH 7.3) at room temperature, incubated with 0.1 M glycine in PBS for 1 h, and rinsed with 0.5% Triton X-100/PBS. Muscles were blocked in the blocking buffer (3% BSA, 5% goat serum, and 0.5% Triton X-100 in PBS) for 2-4 h at room temperature or overnight at 4°C. They were then incubated with primary antibodies ([neurofilament, 1:1000, AB1983, Chemicon](#); [synaptophysin, 1:2000, A0010, Dako](#); [SV2 \(1:1000, Developmental Studies Hybridoma Bank](#); [S100, 1:1000, Z0311, Dako](#); or [rapsyn, 1:1000](#)) in the blocking buffer overnight at 4°C. After washing three times for 1 h each in 0.5% Triton X-100 in PBS, the muscles were incubated with fluorescein-conjugated anti-rabbit or mouse IgG (1:500, Molecular Probes) and Texas Red conjugated  $\alpha$ BTX ([TR  \$\alpha\$ BTX 1:2500, Molecular Probes](#)) 2-4 h at room temperature or overnight at 4°C. Samples were washed three times for 1 h each in 0.5% Triton X-100 in PBS, rinsed once with PBS, and flat-mounted in Vectashield mounting medium (H-1000, Vector laboratories).