Materials List for:
Zebrafish In Situ Spinal Cord Preparation for Electrophysiological Recordings from Spinal Sensory and Motor Neurons

Rosa L. Moreno¹, Megan Josey², Angeles B. Ribera¹,²
¹Department of Physiology and Biophysics, University of Colorado Anschutz Medical Campus (UCAMC)
²Neuroscience Graduate Program, University of Colorado Anschutz Medical Campus (UCAMC)

Correspondence to: Rosa L. Moreno at Rosa.Moreno@ucdenver.edu

URL: https://www.jove.com/video/55507
DOI: doi:10.3791/55507

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Catalog Number</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum filter/Storage bottle, 0.22 mm pore</td>
<td>Corning</td>
<td>431096</td>
<td></td>
</tr>
<tr>
<td>Syringe filter 0.2 mm</td>
<td>Whatman</td>
<td>6780-2502</td>
<td></td>
</tr>
<tr>
<td>Tricaine</td>
<td>Sigma</td>
<td>A-5040</td>
<td>Ethyl 3-aminobenzoate methanesulfonate salt</td>
</tr>
<tr>
<td>α-bugarotoxin</td>
<td>Tocris</td>
<td>11032-79-4</td>
<td></td>
</tr>
<tr>
<td>Tetrodotoxin</td>
<td>Tocris</td>
<td>4368-28-9</td>
<td></td>
</tr>
<tr>
<td>Alexa-549 hydrazine salt</td>
<td>Molecular Probes</td>
<td>A-10438</td>
<td>fluorescent dye</td>
</tr>
<tr>
<td>Spin-X centrifuge tube filter</td>
<td>Corning</td>
<td>8161</td>
<td></td>
</tr>
<tr>
<td>Glass microscope slide</td>
<td>Fisher</td>
<td>12-550C</td>
<td></td>
</tr>
<tr>
<td>Sylgard silicone elastomer kit</td>
<td>Dow Corning</td>
<td>184</td>
<td>silicone elastomer</td>
</tr>
<tr>
<td>Petri dishes</td>
<td>Falcon</td>
<td>351029</td>
<td></td>
</tr>
<tr>
<td>Borosilicate glass capillaries</td>
<td>Harvard Apparatus</td>
<td>30-0038</td>
<td>inner and outer diameters of 0.78 and 1.0 mm (thin walled glass capillaries)</td>
</tr>
<tr>
<td>Borosilicate glass capillaries</td>
<td>Drummond Scientific</td>
<td>1-000-1000-100</td>
<td>inner and outer diameters of 1.13 and 1.55 mm (thick walled glass capillaries)</td>
</tr>
<tr>
<td>Miniature barbed polypropylene fitting</td>
<td>Cole-Palmer</td>
<td>6365-90</td>
<td></td>
</tr>
<tr>
<td>Vetbond tissue adhesive</td>
<td>3M</td>
<td>1469SB</td>
<td></td>
</tr>
<tr>
<td>Collagenase XI</td>
<td>Sigma</td>
<td>C7657</td>
<td></td>
</tr>
<tr>
<td>Microelectrode puller</td>
<td>Sutter Instruments</td>
<td>Model P-97</td>
<td></td>
</tr>
<tr>
<td>Amplifier</td>
<td>Molecular Devices</td>
<td>Axopatch 200B</td>
<td></td>
</tr>
<tr>
<td>Head stage</td>
<td>Molecular Devices</td>
<td>CV203BU</td>
<td></td>
</tr>
<tr>
<td>Motorized micromanipulator</td>
<td>Sutter Instruments</td>
<td>MP-285</td>
<td></td>
</tr>
<tr>
<td>Tygon tubing</td>
<td>Fisher</td>
<td>14-169-1B</td>
<td>ID 1/16 IN, OD 1/8 IN and WALL 1/32 IN (flexible laboratory tubing)</td>
</tr>
<tr>
<td>Electrode holder</td>
<td>Molecular Devices</td>
<td>1-HC-U</td>
<td></td>
</tr>
<tr>
<td>Pharmaseal Three-Way Stopcocks</td>
<td>Baxter</td>
<td>K75</td>
<td></td>
</tr>
<tr>
<td>Digitizer</td>
<td>Axon Instruments</td>
<td>Digidata 1440A</td>
<td></td>
</tr>
<tr>
<td>Inverted microscope</td>
<td>Zeiss</td>
<td>Axioskop2 FS plus</td>
<td></td>
</tr>
<tr>
<td>40X/0.80W Achroplan objective</td>
<td>Zeiss</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Data acquisition and analysis software</td>
<td>Axon Instruments</td>
<td>PClamp 10 - Clampex and Clampfit</td>
<td></td>
</tr>
<tr>
<td>Micropipette puller</td>
<td>Sutter Instruments</td>
<td>Model P-97</td>
<td></td>
</tr>
</tbody>
</table>
### Dissection and Recording Solutions (in mM)

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Catalog Number</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>All solutions, except the intracellular, are stable for ~2-3 months when filtered (0.22 mm filter cups) and stored at room temperature (RT).</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>The intracellular solution is filtered (0.2 mm syringe filters) and stored frozen (-20 °C) in small aliquots that are individually thawed on the day of use.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dissection/Ringer’s solution</td>
<td></td>
<td>145 NaCl, 3 KCl, 1.8 CaCl₂.2H₂O, 10 HEPES; pH 7.4 (with NaOH)</td>
<td></td>
</tr>
<tr>
<td>Pipette (intracellular) recording solution</td>
<td></td>
<td>135 KCl, 10 EGTA-acid, 10 HEPES; pH 7.4 (with KOH).</td>
<td></td>
</tr>
<tr>
<td>Bath (extracellular) recording solution/voltage and current-clamp</td>
<td></td>
<td>125 NaCl, 2 KCl, 10 CaCl₂.2H₂O, 5 HEPES; pH 7.4 (with NaOH).</td>
<td></td>
</tr>
<tr>
<td>Alexa-594 hydrazine salt stock solution</td>
<td></td>
<td>Prepare a 13.2 mM stock in ddH₂O, aliquot (~100 µl) and store at -20 °C. For use, dilute the stock solution 132 fold with pipette solution to a final concentration of 100 mM. After dilution, filter the Alexa-594 containing pipette solution with a centrifuge tube filter.</td>
<td></td>
</tr>
</tbody>
</table>

### Immobilizing agents

<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Catalog Number</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.4% ethyl 3-aminobenzoate methanesulfonate salt (Tricaine)</td>
<td></td>
<td>Prepare a 0.4% stock solution in 0.2 M Tris, pH 9 (0.4 g Tricaine/100 mL 0.2 M Tris)</td>
<td></td>
</tr>
<tr>
<td>Adjust pH to 7 with NaOH and store at -20 °C.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>For use, dilute the stock solution ~25 fold in embryo media</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>250 mM α-bungarotoxin</td>
<td></td>
<td>Prepare a 250 mM stock in ddH₂O (1 mg/500 mL), prepare 100 µL aliquots, and store at -20 °C.</td>
<td></td>
</tr>
<tr>
<td>For use, dilute 2,500-fold with extracellular solution to a final concentration of 100 nM.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mM Tetrodotoxin</td>
<td></td>
<td>Prepare a 1 mM stock in ddH₂O (1 mg/3 mL), prepare 100 µL aliquots, and store at -20 °C.</td>
<td></td>
</tr>
<tr>
<td>For use, dilute 2,000-fold with extracellular solution to a final concentration of 500 nM.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>