Materials List for:

Zebrafish *In Situ* Spinal Cord Preparation for Electrophysiological Recordings from Spinal Sensory and Motor Neurons

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Materials

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

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Dissection and Recording Solutions (in mM)			
All solutions, except the intracellular, are stable for ~2-3 months when filtered (0.22 mm filter cups) and stored at room temperature (RT).			
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.			
Dissection/Ringer's solution			145 NaCl, 3 KCl, 1.8 $CaCl_2.2H_2O$, 10 HEPES; pH 7.4 (with NaOH)
Pipette (intracellular) recording solution			135 KCl, 10 EGTA-acid, 10 HEPES; pH 7.4 (with KOH).
Bath (extracellular) recording solution/voltage and current-clamp			125 NaCl, 2 KCl, 10 $CaCl_2.2H_2O$, 5 HEPES; pH 7.4 (with NaOH).
Alexa-594 hydrazine salt stock solution.			Prepare a 13.2 mM stock in ddH_2O, aliquot (~100 µl) and store at -20 °C. For use, dilute the stock solutiond 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.
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Immobilizing agents			
0.4% ethyl 3-aminobenzoate methanesulfonate salt (Tricaine)			Prepare a 0.4% stock solution in 0.2 M Tris, pH 9 (0.4 g Tricaine/100 mL 0.2 M Tris
Adjust pH to 7 with NaOH and store at -20 °C.			
For use, dilute the stock solution ~25 fold in embryo media			
250 mM α-bungarotoxin			Prepare a 250 mM stock in ddH_2O (1 mg/500 mL), prepare 100 µL aliquots, and sotre at -20 °C.
For use, dilute 2,500-fold with extracellular solution to a final concentration of 100 nM.			
1 mM Tetrodotoxin			Prepare a 1 mM stock in ddH2O (1 mg/3 mL), prepare 100 µL aliquots, and sotre at -20 °C.
For use, dilute 2,000-fold with extracellular solution to a final concentration of 500 nM.			