

Materials List for:

# In Vivo Calcium Imaging of Lateral-line Hair Cells in Larval Zebrafish

Daria Lukasz<sup>1,2</sup>, Katie S. Kindt<sup>1</sup>

<sup>1</sup>Section on Sensory Cell Development and Function, NIDCD/National Institutes of Health

<sup>2</sup>National Institutes of Health-Johns Hopkins University Graduate Partnerships Program

Correspondence to: Katie S. Kindt at [katie.kindt@nih.gov](mailto:katie.kindt@nih.gov)

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## Materials

Name	Company	Catalog Number	Comments
<b>Section 1</b>			
α-Bungarotoxin	R&D Systems	2133	For paralyzing larvae prior to imaging
Phenol red Solution 0.5%	Sigma-Aldrich	P0290	For visualization of α-bungarotoxin solution during heart injection
Ethyl 3-aminobenzoate methanesulfonate (MESAB, MS-222, tricaine)	Sigma-Aldrich	A5040	For anesthetizing larvae
<b>Section 2</b>			
Imaging chamber	Siskiyou	PC-R	Platform to mount larvae for imaging
No. 1.5 Coverslips square, 22*22 mm	VWR	48366-227	To seal imaging chamber
High vacuum silicone grease	Fischer Scientific	14-635-5D	For affixing of coverslip to imaging chamber
Silicone encapsulant clear 0.5 g kit	Ellsworth Adhesives	Dow Corning Sylgard, 184 SIL ELAST KIT 0.5KG	To fill imaging chamber to create a surface to pin fish
Oven	Techne	HB-1D	For drying silicone encapsulant
Stereomicroscope	Carl Zeiss Microscopy	Stemi 2000 with transmitted light illumination	For illuminating wire, forceps and scissors to make pins
Fine forceps	Fine Science Tools	Dumont #5 (0.05 x 0.02 mm) Item No. 11295-10	For making pins
Fine scissors	Cole-Palmer	5.5", EW-10818-00	For cutting tungsten wire to make pins
Tungsten wire, 0.035 mm	Goodfellow	W005131	For head pins to immobilize larvae
Tungsten wire, 0.025 mm	ThermoFischer Scientific	AA10405-H4	For tail pins to immobilize larvae
<b>Section 3</b>			
Micropipette guller	Sutter Instrument Company	P-97	For pulling capillary glass for fluid-jet and heart injection needles
Borosilicate glass capillaries w/ filament	Sutter Instrument Company	BF 150-86-10	Glass to be pulled into α-bungarotoxin injection needles for heart injection
Borosilicate glass capillaries w/o filament	Sutter Instrument Company	B 150-86-10	Glass to be pulled into fluid-jet needles to stimulate hair cells
Pipette polisher	Narishige	MF-830 microforge	To polish fluid-jet pipette tips to smooth jagged breaks
Ceramic tile	Sutter Instrument Company	NC9569052	For scoring and evening breaking fluid-jet needles
<b>Sections 4 &amp; 5</b>			

Fine forceps	Fine Science Tools	Dumont #5 (0.05 x 0.02 mm) Item No. 11295-10	For pinning larvae
Gel loading tips	Eppendorf	5242956003	For backfilling heart injection needles
Stereomicroscope	Carl Zeiss Microscopy	Stemi 2000 with transmitted light illumination	For illuminating larvae during pinning and heart injection
Glass capillary/needle holder	WPI	MPH315	To hold fluid-jet needles
Manual micromanipulator	Narishige	M-152	For holding and positioning of needle holder to inject $\alpha$ -bungarotoxin
Magnetic stand	Narishige	GJ-1	For holding manual micromanipulator for $\alpha$ -bungarotoxin injection
Pressure injector	Eppendorf	Femtojet 4x	To deliver $\alpha$ -bungarotoxin
<b>Sections 6, 7 &amp; 8</b>			
Confocal microscope	Bruker	Swept field/Opterra confocal microscope	Fixed, upright microscope with DIC optics and 488 nm laser with appropriate filters
Microscope software	Bruker	Prairieview 5.3	To coordinate and control the microscope, lasers, stage, piezo-z, cameras and fluid jet
10x air objective	Nikon	MRH00101	Low magnification for positioning of larvae and fluid jet
60x water objective	Nikon	MRF07620	Water immerison objective with high NA (1.0) and adequate working distance (2.0 mm)
Piezo-Z objective scanner with controller/driver	Physik Intruments instruments/ Bruker	01144210/UM-Z-PZ	High-speed z-stack acquisition with 0.025um accuracy
EMCCD camera	QImaging	Rolera EM-C2 EMCCD camera	Camera with small pixel size that can acquire up to 100 frames per s
Circular chamber adapter	Siskiyu	PC-A	For holding and rotating imaging chamber on microscope stage
Motorized Z-deck stage	Prior Scientific	ZDN12MP	Microscope stage that can hold and move sample and micromanipulator with fluid-jet needle together
Z-deck stage insert adaptor	NIH Machine shop	custom	To fit the circular chamber adaptor onto the z-deck stage
Fluid-jet apparatus	ALA scientific instruments	HSPC-1 High-speed pressure Clamp with PV-PUMP	For controlling and delivering the fluid-jet stimulus
Masterflex Peroxide-cured silicone tubing (1 feet)	Cole-Palmer	Masterflex L/S 13, 96400-13	For connecting the fluid-jet needle holder to pressure pump
Motorized micromanipulator	Sutter Instrument Company	MP-225	For holding and positioning of needle holder for fluid jet
Micromanipulator controller	Sutter Instrument Company	MPC-200	For controlling fluid-jet needle manipulator
Gel loading tips	Eppendorf	5242956003	For backfilling fluid-jet needles
Glass capillary/needle holder	WPI	MPH315	To hold fluid-jet needles
PSI manometer	Sper Scientific	840081	For measuring pressure clamp output
<b>Section 9</b>			
BAPTA, Tetrapotassium Salt, cell impermeant	ThermoFischer Scientific	B1204	To cleave tips links, uncouple MET channels and block all evoked GCaMP6s signals

Isradipine	Sigma-Aldrich	I6658	To block signals dependent on the L-type calcium channels (CaV1.3) at the presynapse
DMSO	Sigma-Aldrich	D8418	Solvent for pharmacological compounds
Section 10			
Prism7	Graphpad	Prism7	Software to plot GCaMP6 intensity changes
FIJI	Schindelin, et., al. <sup>34</sup>	<a href="https://fiji.sc/">https://fiji.sc/</a>	Software to process images and create spatio-temporal signal maps
Turboreg Plugin	Thévenaz et., al. <sup>29</sup>	<a href="http://bigwww.epfl.ch/thevenaz/turboreg/">http://bigwww.epfl.ch/thevenaz/turboreg/</a>	Plugin to register GCaMP6 image sequences in FIJI
StackReg Plugin	Thévenaz et., al. <sup>29</sup>	<a href="http://bigwww.epfl.ch/thevenaz/stackreg/">http://bigwww.epfl.ch/thevenaz/stackreg/</a>	Plugin to register GCaMP6 image sequences in FIJI
Times Series Analyzer V3 Plugin	Balaji J 2007, Dept. of Neurobiology, UCLA	<a href="https://imagej.nih.gov/ij/plugins/time-series.html">https://imagej.nih.gov/ij/plugins/time-series.html</a>	Plugin to create multiple ROIs to measure GCaMP6 intensity changes