

Materials List for:

# Ex Vivo Imaging of Cell-specific Calcium Signaling at the Tripartite Synapse of the Mouse Diaphragm

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## Materials

Name	Company	Catalog Number	Comments
Myf5-Cre mice	Jax	#007893	Drives muscle cell expression as early as E13 <sup>6</sup>
Wnt1-Cre mice	Jax	#003829	Drives expression into all Schwann cells at E13 but not P20 <sup>9</sup>
Sox10-Cre mice	Jax	#025807	Drives Schwann cell expression at older ages
Conditional GCaMP3 mice	Jax	#029043	Expresses GCaMP3 in cell-specific fashion
Conditional GCaMP6f mice	Jax	#024105	Expresses GCaMP6f in cell-specific fashion
BHC (3-(N-butylethanimidoyl)-4-hydroxy-2H-chromen-2-one)	Hit2Lead	#5102862	Blocks skeletal muscle myosin but not neurotransmission <sup>6</sup>
CF594- $\alpha$ -BTX	Biotium	#00007	Labels acetylcholine receptor clusters at NMJ
$\mu$ -conotoxin GIIIB	Peptides Int'l	#CONO20-01000	Blocks Nav1.4 voltage-dependent sodium channel <sup>8</sup>
Silicone Dielectric Gel; aka Sylgard	Ellsworth Adhesives	# Sil Dielec Gel .9KG	Allows for the immobilization of the diaphragm by minuten pins
Minutien pins (0.1mm diameter)	Fine Science Tools	26002-10	Immobilizes diaphragm onto silicone dielectric gel
Eclipse FN1 upright microscope	Nikon	MBA74100	Allows staging and observation of specimen
Basic Fixed Microscope Platform with Manual XY Microscope Translator	Autom8	MXMScr	Allows movement of specimen
Manual micromanipulator	Narishige	M-152	Holds recording and stimulating electrodes
Microelectrode amplifier	Molecular Devices	Axoclamp 900A	Allows sharp electrode intracellular electrophysiological recording
Microelectrode low-noise data acquisition system	Molecular Devices	Digidata 1550	Allows electrophysiological data acquisition
Microelectrode data analysis system	Molecular Devices	PCLAMP 10 Standard	Performs electrophysiological data analysis
Square wave stimulator	Grass	S48	Stimulates nerve to excite muscle
Stimulus Isolation Unit	Grass	PSIU6	Reduces stimulation artifacts
Borosilicate filaments, 1.0 mm outer diameter, 0.5mm internal diameter	Sutter	FG-GBF100-50-15	Impales and records nerve-evoked muscle potentials

Borosilicate filaments, 1.5 mm outer diameter, 1.17mm internal diameter	Sutter	BF150-117-15	Lengthened and used for suction electrode
Micropipette Puller	Sutter	P-97	Pulls and prepares recording electrodes
1200x1200 pixel, back-illuminated CMOS camera	Photometrics	Prime 95b	Sensitive camera that allows high-resolution, high-speed imaging
Light Source	Lumencor	Spectra X	Provides illumination from LEDs for fluorescence observation
Infinity-corrected fluorescent water immersion objectives, W.D. 2mm	Nikon	CFI60	Provide long working distances for visualization of specimen
Fiber Optic Illuminator with Halogen lamp	Sumita	LS-DWL-N	Provides illumination for brightfield observation
W-View Gemini Image Splitter	Hamamatsu	A12801-01	Projects 1 pair of dual wavelength images separated by a dichroic to single camera
Single-band Bandpass Filters (512/25-25 and 630/92-25)	SemRock	FF01-512/25-25; FF01-630/92-25	Permits dual band imaging
560 nm Single-Edge Dichroic Beamsplitter	Sem Rock	FF560-FDi01-25x36	Dichroic mirror which separates beams of light to allow dual-wavelength imaging
Imaging data acquisition system	Nikon	NIS Elements - MQS31000	Allows imaging data acquisition
Wavelength control module	Nikon	MQS41220	Module for imaging data acquisition
Emission splitter hardware module	Nikon	MQS41410	Module for imaging data acquisition
Imaging data analysis system	NA	Volumetry 8D <sup>5</sup> , Fiji	Allows analysis of fluorescence intensity and other imaging data