

Materials List for:

Isolation of Retinal Arterioles for *Ex Vivo* Cell Physiology Studies

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Materials

Name	Company	Catalog Number	Comments
Beakers	Fisherbrand	15409083	Or any equivalent product
Curved Scissors	Fisher Scientific	50-109-3542	Or any equivalent product
Disposable plastic pipette/ transfer	Sarstedt	86.1174	6mL is best but other sizes are acceptable; remove tip to widen aperture
Dissecting microscope	Brunel Microscopes LTD		Or any equivalent product
Forceps	World Precision Instruments	Dumont #5 14095	Any equivalent fine forceps
Pasteur pipette	Fisherbrand	11546963	Fire polished to reduce friction but not enough to narrow the tip
Petri dish	Sigma-Aldrich	P7741	Or any equivalent 10cm product
Pipette teat	Fisherbrand	12426180	Or any equivalent product
Purified water supply	Merek	Milli-Q Integral Water Purification System	Or any equivalent system
Round bottomed test tube	Fisherbrand	14-958-10 B	5 mL; any equivalent product
Serrated forceps	Fisher Scientific	17-467-230	Or any equivalent product
Single edge blades	Agar Scientific	T585	Or any equivalent product
Sylgard	Dow Corning	184	Or any pliable surface
Testtube rack	Fisherbrand Derlin	10257963	Or any equivalent product
Pressure myography			
3-axis mechanical manipulator	Scientifica	LBM-7	x2 (or any equivalent product)
3-way taps	Cole-Parmer	UY-30600-02	Or any equivalent product
Air table	Technical Manufacturing Corporation	Clean Bench	Or any equivalent product
Analysis software	Image J	https://downloads.imagej.net/fiji/	Free imaging software
Analysis plugin	Myotraker	https://doi.org/10.1371/journal.pone.0091791.s002	Custom plugin for imageJ Freely available for download
Cannulation pipette glass	World Percision instruments	TW150F-4	Or any equivalent product
Computer	Dell	Optiplex 7010	Or any equivalent product
Digital Thermometer	RS	206-3722	Or any equivalent product
Fluo-4-AM	Thermo Fisher Scientific	F14201	Make 1 mM stock in DMSO
Forceps	World Precision Instruments	Dumont #7 14097	Any equivalent fine forceps
Fura-2-AM	Thermo Fisher Scientific	F1221	Make 1 mM stock in DMSO
Glass bottomed recording chamber	Warner Instruments	64-0759	Or any equivalent product

Helper pipette glass	World Percision instruments	IB150F-3	Or any equivalent product
In-line heater	Custom made		Commerical equivalent SH-27B Solution In-Line Heater from Harvard Apparatus
Inverted microscope	Nikon	Eclipse TE 300	Or any equivalent product
Manometer	Riester	LF1459	Or any equivalent product
Microelectrode puller	Sutter instruments	P97	Or any equivalent product
Microforge + Olympus CX 31 microscope	Glassworks	Fine Point F-550	Or any equivalent product
Micromanipulator	Sutter instruments	MP-285	Or any equivalent product
Multi-channel delivery manifold	Automate Scientific	Perfusion Pencil	Or any equivalent product
Pipette filler	BD Plastipak	1mL	Syringe heated and pulled to internal diameter of pipette
Pipette holder	Molecular Devices	1-HL-U	Or any equivalent product
Suction pump	Interpet	AP1	Converted aeration pump by reversing bellows
Syringe	BD Plastipak	20mL Luer-lok	Or any equivalent product
Tungsten wire	Advent	W558818	75µm diameter
Tygon Tubing	VWR	miscellaneous sizes	Or any equivalent product
USB camera	Logitech	HD Pro Webcam C920	Or any equivalent product
Video capture software	Hypercam	version 2.28.01	Or any equivalent product
Water bath	Grant	Sub Aqua 12 Plus	Or any equivalent product
x20 lens	Nikon	20x/0.40 WD 3.8, ∞/0.17	Or any equivalent product
x4 lens	Nikon	4x/0.10, WD 30, ∞/-	Or any equivalent product
Y-connectors	World Percision Instruments	14012	Or any equivalent product
Patch clamping			
3-way taps	Cole-Parmer	UY-30600-02	Or any equivalent product
3-axis mechanical manipulator	Scientifica	LBM-7	Or any equivalent product
Air table	Technical Manufacturing Corporation	Clean Bench	Or any equivalent product
Amphotericin B	Sigma-Aldrich	A2411	3 mg dissolved daily in 50 µL of DMSO (sonicate to dissolve)
Amplifier	Molecular Devices	Axopatch 200a	Or any equivalent product
Analogue digital converter	Axon	Digidata 1440A	Or any equivalent product
Collagenase Type 1A	Sigma-Aldrich	C9891	0.1mg/mL in LCH
Computer	Dell	Optiplex 7010	Or any equivalent product
Digital Thermometer	RS	206-3722	Or any equivalent product
DNAse I	Millipore	260913	Working stock 1 MU mL ⁻¹ (10 MU diluted in 10 mL LCH)
Faraday cage	Custom made		Any equivalent commercial product
Fine forceps	Dumont	No. 7	Any superfine forcep
Glass bottomed recording chamber	Warner Instruments	64-0759	Or any equivalent product
Headstage	Molecular Devices	CV203BU	Or any equivalent product
In-line heater	Custom made		Commerical equivalent SH-27B Solution In-Line Heater from Harvard Apparatus
Inverted microscope	Nikon	Eclipse TE 300	Or any equivalent product
Microelectrode puller	Sutter instruments	P97	Or any equivalent product

Microforge + Olympus CX 31 microscope	Glassworks	Fine Point F-550	Or any equivalent product
Micromanipulator	Sutter instruments	MP-285	Or any equivalent product
Multi-channel delivery manifold	Automate Scientific	Perfusion Pencil	Or any equivalent product
Patching software	Molecular Devices	Pclamp v10.2	Or any equivalent product
Pipette filler	BD Plastipak	1mL	Syringe heated and pulled to internal diameter of pipette
Pipette holder	Molecular Devices	1-HL-U	Or any equivalent product
Protease Type XIV	Sigma-Aldrich	P5147	0.01mg/mL in LCH
Single channel pipette glass	World Percision instruments	IB150F-3	Or any equivalent product
Suction pump	Interpet	AP1	Converted aeration pump by reversing bellows
Syringe	BD Plastipak	20mL Luer-lok	Or any equivalent product
Tungsten wire	Advent	W557418	50µm diameter
Tygon Tubing	VWR	miscellaneous sizes	Any equivalent product to fit
USB camera	Logitech	HD Pro Webcam C920	Or any equivalent product
Water bath	Grant	Sub Aqua 12 Plus	Or any equivalent product
Whole cell pipette glass	Warner Instruments	GC150TF-7.5	Or any equivalent product
x20 lens	Nikon	20x/0.40 WD 3.8, ∞/0.17	Or any equivalent product
x4 lens	Nikon	4x/0.10, WD 30, ∞/-	Or any equivalent product
x40 lens	Nikon	40x/0.55, WD 2.1, ∞/1.2	Or any equivalent product
Y-connectors	World Percision Instruments	14012	Or any equivalent product
Ca ²⁺ imaging			
3-way taps	Cole-Parmer	UY-30600-02	Or any equivalent product
3-axis mechanical manipulator	Scientifica	LBM-7	Or any equivalent product
Acquisition software	Cairn Research Ltd.	Acquisition Engine V1.1.5	Or any equivalent product; microfluorimetry
Air table	Technical Manufacturing Corporation	Clean Bench	Or any equivalent product
Analysis software for confocal imaging	Image J	https://downloads.imagej.net/fiji/	Free imaging software
Computer	Dell	Optiplex 7010	Or any equivalent product
Confocal microscope	Leica Geosystems	SP5	Or any equivalent product; confocal
Digital Thermometer	RS	206-3722	Or any equivalent product
Fine forceps	Dumont	No. 7	Any superfine forcep
Glass bottomed recording chamber	Warner Instruments	64-0759	Or any equivalent product
In-line heater	Custom made		Commerical equivalent SH-27B Solution In-Line Heater from Harvard Apparatus
Inverted microscope	Nikon	Eclipse TE2000	Or any equivalent product; microfluorimetry
Monochromator	Cairn Research Ltd.	Optoscan	Or any equivalent product; microfluorimetry
Multi-channel delivery manifold	Automate Scientific	Perfusion Pencil	Or any equivalent product
Pipette filler	BD Plastipak	1mL	Syringe heated and pulled to internal diameter of pipette
Software for confocal microscope	Leica Geosystems	LAS-AF version 3.3.	Or any equivalent product; confocal

Suction pump	Interpet	AP1	Converted aeration pump by reversing bellows
Syringe	BD Plastipak	20mL Luer-lok	Or any equivalent product
Tungsten wire	Advent	W557418	50µm diameter
Tygon Tubing	VWR	miscellaneous sizes	Any equivalent product to fit
Water bath	Grant	Sub Aqua 12 Plus	Or any equivalent product
x100 lens	Nikon	x100 N.A. 1.3 oil	Or any equivalent product; microfluorimetry
x20 lens	Nikon	20x/0.40 WD 3.8, ∞/0.17	Or any equivalent product; microfluorimetry
x20 lens	Leica Geosystems	HCX PL FLUOTAR, 20x/0.50, ∞/0.17/D	Or any equivalent product; confocal
x4 lens	Nikon	4x/0.10, WD 30, ∞/-	Or any equivalent product; microfluorimetry
x63 lens	Leica Geosystems	HCX PL APO, 63x/1.40 - 0.60 OIL CS ∞/0.17/E	Or any equivalent product; confocal
Y-connectors	World Percision Instruments	14012	Or any equivalent product
Pipettes			Specifications and settings for fabrication of pipettes for experimentation
Helper pipette	World Percision instruments	IB150F-3	Inner diameter: 0.86 mm Ramp: 261 Pressure: 300 Heat: 280 Pull : 0 Velocity: 56 Time: 250 No of cycles: 4 Tip diameter: 0.5-2 µm Polishing: yes Final Resistance:>10 MΩ
Cannulation pipette	World Percision instruments	TW150F-4	Inner diameter: 1.17 mm Ramp: 290 Pressure: 300 Heat: 280 Pull : 0 Velocity: 58 Time: 150 No of cycles: 4 Tip diameter: 3-10 µm Polishing: no Final Resistance: <1 MΩ
On-cell patching	World Percision instruments	IB150F-3	Inner diameter: 0.86 mm Ramp: 261 Pressure: 300 Heat: 280 Pull : 0 Velocity: 56 Time: 250 No of cycles: 4 Tip diameter: 0.5-2 µm Polishing: yes Final Resistance:>5 MΩ
Whole-cell patching	Warner Instruments	GC150TF-7.5	Inner diameter: 1.17 mm Ramp: 296 Pressure: 200 Heat: 287 Pull : 0 Velocity: 50 Time: 250 No of cycles: 4

			Tip diameter: 2-3 μm Polishing: helpful but not necessary Final Resistance: 1-2 M Ω
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