Materials List for:

Modeling Neural Immune Signaling of Episodic and Chronic Migraine Using Spreading Depression *In Vitro*

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URL: http://www.jove.com/video/2910/

Materials

Name	Company	Catalog Number	Comments
Basal Medium Eagle	Invitrogen	21010	
Earle's Balanced Salt Solution	Sigma	E2888	
Horse Serum	Invitrogen	26050-088	
Glutamax	Invitrogen	35050	
Gentamicin	Invitrogen	15710-064	
Fungizone	Invitrogen	15295	
D-glucose	Sigma	G8769	

Table 1. Culture Preparation and Maintenance: Horse Serum Medium

Name	Company	Catalog Number	Comments
Neurobasal	Invitrogen	21103	
Gem-21 Neuroplex	Gemini Bioproducts	400-160-010	
Glutamax	Invitrogen	35050	
Gentamicin	Invitrogen	15710-064	
D-glucose	Sigma	G8769	
Ascorbic acid	Sigma	A4544	
Fungizone	Invitrogen	15295	
Sodium chloride	Sigma	S6546	
Magnesium chloride	Sigma	M1028	
Calcium chloride	Sigma	21115	

Table 2. Culture Preparation and Maintenance: Serum-free Medium

Name	Company	Catalog Number	Comments
Millicell-CM tissue culture inserts (30 mm)	Thermo Fisher	PICM0-3050	
6-well Falcon culture dishes	Thermo Fisher	08-772-1B	
Sytox Green	Invitrogen	S7020	

Table 3. Materials fabrication: Ground Electrodes

Name	Company	Catalog Number	Comments
Glass capillary tubes	A-M Systems, Inc.		2mm x 1.16mm, 4 inches
KCl	Sigma	P5405	
Agar	Fisher Scientific	A360	
EDTA	Sigma	5134	

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Tubing	Masterflex	95809-34	
1.5 mL centrifuge tube rack	Fisher Scientific		
Silver wire	Medwire	AG15X	
Hemostat	Fine Scientific Tools	13018-14	
Emery board	Walgreens		
Scintillation vial	Fisher Scientific		
Flexible adhesive sealant	Amazing Goop		
Bleach	Clorox		

Table 4. Materials fabrication: Ground Electrodes

Name	Company	Catalog Number	Comments
Borosilicate filament	Sutter Instruments	BF150-86-10	1.5 x 0.86 mm
Microelectrode puller	Narashige	PE-2	
Eye piece optical micrometer	Zeiss		
Stage optical micrometer	Zeiss		
Compound Microscope	Zeiss		
Microscope slides	Surgipath	00210	
1-Dimensional microelectrode manipulator	Narashige	MO-10	
3-dimensional microelectrode manipulator	Narashige	MM-3	
Adhesive putty	Scotch		
100 mm Nalgene containers	Fisher Scientific		

Table 5. Materials fabrication: Recording Electrodes

Name	Company	Catalog Number	Comments
Teflon-coated platinum iridium wire	A-M Systems	7780	125 µm bare, 200 µm coated
Forceps	Thermo Fisher	13-812-38	
Hemostat	Fine Scientific Tools	13018-14	
30 gauge wire	Tensolite		
Soldering iron	Cooper Tools	UTC 100	
Solder	Bernzomatic		Resin core, lead free, silver bearing
Concentrated HCl	Fisher Scientific	A144-212	
Pasteur pipette	Fisher Scientific	13-678-30	
Water-resistant epoxy	JB Weld		
Banana jacks	Newark Electronics		

Table 6. Materials fabrication: Stimulating Electrodes

Name	Company	Catalog Number	Comments
Falcon 35 mm culture dish	Becton Dickinson Labware		
Glass filament tubes	Sutter Instrument Co.	BF100-58-10	1.0 mm, 12 mm long
Cotton squares	Walgreens		
Forceps	Thermo Fisher	13-812-38	
Tubing	Masterflex	95809-34	
Poly vinyl chloride film	Fisher Scientific	01810	18 inches x 1000 feet
#9 single edge razor blade	Smith		

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Table 7. Materials fabrication: Recording Dishes

Name	Company	Catalog Number	Comments
Recording chamber	Medical Systems Corp.	PDMI-2	Recording chamber
Bipolar temperature control	Medical Systems Corp.	TC-202	
Combination pH electrode	Microelectrodes Inc.	MI-413	
pH meter	Beckman	250	Battery operated
Mini Type-T thermocouples	Physiotemp		IT series
DigiSense thermometer	Cole-Palmer	8528-10	
Inline heater	Warner Instruments	SH278	
Gibraltor table	Burleigh		
Inverted microscope	Leica	DMIRE2	
Anti-vibration Table	Technical Manufacturer's Corp	63-541	
Cautery tool	Gemini		Battery operated
Microelectrode micromanipulators	Narashige	WR60	Left and right
Stabilized power supply	MGE UPS systems	Ellipse 1200	+/- 5 volts (120 total)
Axoprobe amplifier system	Axon instruments	1-A	
Active filter	Frequency Devices	Model 900	
Axoscope Digidata	Axoscope	1322-A	
Axoscope software	Axoscope	v. 9	
Computer systems	Dell	Precision T5400	
Origin8	OriginLab Corp	v. 8.1	
Stimulator isolation unit	Bak Electronics, Inc.	BSI-II	
1/2 inch position magnets	Dexter	PMO90-3	
3 inch position magnets	Dexter	PMC-800T	
Multiple X-Blocks	Narishige	X-12	For positioning ball joints
Valves	Hamilton	HVD-3	For medium delivery
Syringe pump	Razel	A99	
Digital oscilloscope	Agilant technologies		
Digital camera system	Quant EM	512-SC	
MetaMorph software	Molecular Devices	v. 7.5.04	
Microscope objective focus with Pi foc	Physik Instrumente	E-662.LR	
Smart shutter	Lambda SC		
Dual wavelength fluorescent microscopy	Applied Scientific Instruments	Polychrome II	
Peristaltic pumps	Masterflex	77120-62	
Aspirator	Harvard Apparatus		PDMI component

Table 8. Electrophysiological Setup

Name	Company	Catalog Number	Comments
1 M Magnesium Chloride	Sigma	057K8620	
1 M Manganese Chloride	Sigma	018K6107	
1 M Calcium Chloride	Sigma	GA10984	
AlexaFluor594-tagged Isolectin-IB_4	Invitrogen	I21413	
Neurobasal	Invitrogen	21103	
Polyvinyl chloride film	Fisher Scientific	01810	

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MetaMorph	Molecular Devices	v. 7.5.04	
Inverted microscope	Leica	DMIRE2	
Camera	QuantEM	512SC	
UV light	Kubler	CODIX	
Bipolar Temperature Controller	Medical Systems Corp	TC-202	
Smart shutter	Lambda SC		
Sytox Green	Invitrogen	S7020	
Falcon 35 mm culture dishes	Becton Dickinson Labware		
Glass filament tubes	Sutter Instrument Co.	BF100-58-10	1.0 mm, 12 mm long
Tubing	Masterflex	95809-34	
Recording chamber	Medical Systems Corp.	PDMI-2	Recording chamber
Bipolar temperature control	Medical Systems Corp.	TC-202	
Combination pH electrode	Microelectrodes Inc.	MI-413	
Gibraltor table	Burleigh		
Anti-vibration Table	Technical Manufacturer's Corp	63-541	

Table 9. Vital Imaging in Hippocampal Slice Cultures

Name	Company	Catalog Number	Comments
RNA*later*	Ambion	AM7021	
RNase/DNase-free 1.5 mL microcentrifuge tubes	USA Scientific	1615-5500	
Diamond knife	Fine science tools	10100-00	
Fine-tip paint brush	Ted Pella	11806	
TRIzol	Invitrogen	15596-026	
Centrifuge	Eppendorf	5451C	
Vortex-genie	VWR	G-560	

Table 10. PCR Preparation: Slice Culture Harvest

Name	Company	Catalog Number	Comments
Chloroform	Sigma	C-2432	
Centrifuge	Eppendorf	5451C	in cold room
Ethanol, 200 proof for molecular biology	Sigma	E7023	
RNeasy Micro kit	Qiagen	74004	

Table 11. PCR Preparation: RNA Extraction

Name	Company	Catalog Number	Comments
Nanodrop 2000	Thermo Fisher	ND-2000	
3-(N-morpholino) propanesulfonic acid	Sigma	M-1254	
ssRNA ladder	NEB	N032S	
Sodium acetate	Sigma	S-9513	
EDTA	Sigma	E5134	
Ethidium Bromide solution	Bio-Rad	161-0433	
OWL easycast horizontal mini-gel systems	Thermo Fisher	B1	
Electrophoresis power source	Bio-Rad	PowerPac HC	

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Ultrapure Agarose	GibcoBRL	15510-019	
Microwave	Samsung	SJ0390W	
RNase *Away*	Molecular Bioproducts	7000	

Table 12. PCR Preparation: RNA Quantification

Name	**Company**	**Catalog Number**	**Comments**
iCycler thermocycler	Bio-Rad	iCycler Optical Module	
iCycler system software	Bio-Rad	v. 3.1	
iCycler iQ PCR plates, 96 well	Bio-Rad	2239441	
Flat cap strips - optically clear. 8-cap strip	Bio-Rad	TCS0803	
iScript cDNA synthesis kit	Bio-Rad	170-8890	
iQ SYBR Green supermix	Bio-Rad	170-8880	
Ultrapure Agarose	GibcoBRL	15510-019	
10x TAE buffer	GibcoBRL	15558-026	
Microwave	Samsung	SJ0390W	
RNase *Away*	Molecular Bioproducts	7000	
Ethidium Bromide solution	Bio-Rad	161-0433	
OWL easycast horizontal mini-gel systems	Thermo Fisher	B1	
Electrophoresis power source	Bio-Rad	PowerPac HC	
Name	sequence		
TNF-α F	5' - ACC ACG CTC TTC TGT CTA CTG A- 3'		
TNF-α R	5' - CTG ATG AGA GGG AGC CCA TTT G- 3'		
Rpl13a F	5' - TTG CTT ACC TGG GGC GTC T- 3'		
Rpl13a R	5' - CTT TTT CCT TCC GTT TCT CCT C- 3'		

Table 13. PCR Activities: qPCR primer optimization and pre-screening

Name	**Company**	**Catalog Number**	**Comments**
RT^2 Profiler PCR array	SABiosciences	PARN-021	
RT^2 Nano preAMP cDNA synthesis kit	SABiosciences	C-06	
RT^2 Nano preAMP cDNA synthesis primer mix	SABiosciences	PBR-3021	
RT^2 SYBR Green/ fluorescein qPCR master mix	SABiosciences	PA-011	
RT^2 qPCR Primer Assay for Rat IFN-λ	SABiosciences	PPR45050C	
Multichannel pipettor	Corning		
25 mL Bio-pure reservoir with divider	Diversified biotech	REBP-2000	
iCycler thermocycler	Bio-Rad	iCycler Optical Module	
iCycler system software	Bio-Rad	v. 3.1	

Table 14. qPCR array plates and low level signaling

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Name	Company	Catalog Number	Comments
Centrifuge	Fisher Scientific	Micro 7	
Cell lysis kit	Bio-Rad	171-304011	
BSA protein stock	Bio-Rad	500-0007	
BCA protein assay kit	Thermo Scientific	23225	
Sealing tape	Bio-Rad	2239444	
96-well plate reader	Perkin Elmer	1420-multilabel counter	
Phospho 6-plex Assay Lysates	Bio-Rad	X7000000502	
Phospho 6-plex coupled beads	Bio-Rad	X700000050	

Table 15. Multiplex Phosphoprotein Assay

Name	Company	Catalog Number	Comments
Accell 5x siRNA Buffer	Thermo Scientific	B-002000-UB-100	
Accell siRNA Delivery Medium	Thermo Scientific	B-005000-100	
Accell Cyclophilin Pool-Rat	Thermo Scientific	D-001920-30-05	

Table 16. siRNA

Name	Company	Catalog Number	Comments
16% paraformaldehyde	Alfa Aesar	43368	
Lysine	Sigma	L-6001	
Sodium phosphate	Fisher Scientific	S374-1	
Sodium periodate	Sigma	S-1878	
Sodium azide	Sigma	S-2002	
Cryostat	Leica	CM3050 S	
Tissue-Tek	Ted Pella Inc.	27050	
Fine-tip paint brush	Ted Pella	11806	
Tissue slides	Surgipath	00210	
Coplin jars	Fisher	08-815	
Hydrogen peroxide (30%)	Sigma	H1009	
SFX signal enhancer	Invitrogen	A31631	
Goat serum	Colorado Serum Company	CS 0920	
Triton X-100	Sigma	T-8787	
Anti-NFκβ antibody	Santa Cruz	SC-109	
Goat anti-rabbit IgG Alexa Fluor 594	Invitrogen	A11012	
Yo-yo nuclear counterstain	Invitrogen	Y-3601	
ProLong antifade	Invitrogen	P7481	

Table 17. Proteomic Confirmation Via Immunohistochemistry

Name	Company	Catalog Number	Comments
Anti-cyclophilin B	R&D Systems	MAB5410	
3,3-diaminobenzidine	Sigma	D8001	
Water bath	Pharmacia Biotech	56-1165-33	Gebrüder HAAKE GmbH
Ammonium hydroxide	J.T.Baker	9721-03	
Silver nitrate	Sigma	S-0139	
Sodium thiosulfate (pentahydrate)	J.T.Baker	3949-01	&nsp;

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0.2% gold chloride	Sigma	G-4022	
Oxalic acid	Sigma	O-0376	
CoolSnap *fx* CCD camera	Photmetrics		
MetaMorph software	Molecular Devices	v. 7.5.04	
Inverted microscope	Leica	DM IRBE	
Neutral density filters	Chroma		0.5-3.0 optical density unit range

Table 18. Proteomic Confirmation Via Silver Enhanced Immunohistochemistry

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