

Materials List for:

Monitoring Astrocyte Reactivity and Proliferation *in Vitro* Under Ischemic-Like Conditions

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Materials

Name	Company	Catalog Number	Comments
Instruments for Surgery - Step 1			
Operating scissor 5.5"	Roboz Company	RS-6812	Tools used to decapitate the rats.
Curved forceps 7"	Roboz Company	RS-5271	Holds the skin of the rat while the skull is removed.
Micro-dissecting scissors 4"	Roboz Company	RS-5882	Cuts both the skin and skull of the rat.
Micro-dissecting forceps 4" angled, fine sharp	Roboz Company	RS-5095	Holds the skin of the rat while the skull is removed.
Micro-dissecting forceps 4" slightly curved 0.8	Roboz Company	RS-5135	Tool used to separate cortices.
Micro-dissecting tweezers	Roboz Company	RS-4972	Peels brain meninges.
Dissection microscope	Olympus	SZX16	Important for removing the meninge from the cortices.
DMEM Preparation - step 2			
Dulbecco's Modified Eagle's Medium (DMEM)	GibCo. Company	11995-065	Supports the growth of cells.
Sodium bicarbonate	Sigma-Aldrich Company	S7277	Supplement for the cell culture media.
Fetal bovine serum (FBS)	GibCo. Company	10437-010	Serum-supplement for the cell culture.
Penicillin-Streptomycin	GibCo. Company	15140-148	Inhibits the growth of bacterias in the cell culture.
Filter System 1L with 0.22um pore	Corning	431098	
Astrocyte culture - step 3			
Serological pipets 5mL	VWR	89130-896	To pipette DMEM to containers with cells.
Serological pipets 10mL	VWR	89130-898	To pipette DMEM to containers with cells.
Serological pipets 25mL	VWR	89130-900	To pipette DMEM to containers with cells.
Centrifuge conical tube 15mL	Santa Cruz Biotechnology	sc-200250	
Safe-lock tube 1.5mL	Eppendorf	022363204	
Barrier Tips 200 uL	Santa Cruz Biotechnology	sc-201725	
Barrier Tips 1 mL	Santa Cruz Biotechnology	sc-201727	
Biohazard Orange Bag 14 x 19"	VWR	14220-048	

60mm petri dishes	Falcon	351007	
Sterile gauze pads	Honeywell Safety	89133-086	
Stomacher 80 Biomaster	Sewar Lab System	030010019	Triturate the brain tissue.
Stomacher 80 Blender Sterile Bags	Sewar Lab System	BA6040	Sterile bag for the stomacher cell homogenizer.
Beaker 400mL	Pyrex	1000	
Sterile cell dissociation sieve, mesh #60	Sigma-Aldrich Company	S1020	To obtain a uniform single cell suspension.
Sterile cell dissociation sieve, mesh #100	Sigma-Aldrich Company	S3895	To obtain a uniform single cell suspension.
Invert phase microscope	Nikon	Eclipse Ti-S	Verify cells for contamination or abnormal cell growth.
75cm ² sterile flasks	Falcon	353136	
Multi-well plate	Falcon	353046	
Micro cover glasses (coverslips), 18mm, round	VWR	48380-046	
Bright-Line hemacytometer	Sigma-Aldrich Company	Z359629	
Pasteur pipettes	Fisher Scientific	13-678-20D	
Ethyl alcohol	Sigma-Aldrich Company	E7023	
L-leucine methyl ester hydrochloride 98% (LME)	Sigma-Aldrich Company	L1002	Promotes the elimination of microglia cells in the primary cortical astrocyte culture.
Cytosine β-D-arabinofuranoside (Ara-C)	Sigma-Aldrich Company	C1768	
Poly-D-Lysine Hydrobromide, mol wt 70,000-150,000	Sigma-Aldrich Company	P0899	
Trypsin/EDTA	GibCo. Company	15400-054	
Trypan Blue	Sigma-Aldrich Company	T8154	
Phosphate buffer saline (PBS) tablets	Calbiochem	524650	
Sterile Water	Sigma-Aldrich Company	W3500	
OGD Medium Preparation - step 5			
Centrifuge conical tube 50 mL	VWR	89039-658	
Dulbecco's modified Eagle's medium-free glucose	Sigma-Aldrich Company	D5030	Supports the growth of cells.
Sodium bicarbonate	Sigma-Aldrich Company	S7277	Supplement for the cell culture media.
Penicillin-Streptomycin	GibCo. Company	15140-148	Inhibits the growth of bacterias in the cell culture.
200mM L-glutamine	GibCo. Company	25030-081	Amino acid that supplements the growth of cells.
Phosphat buffer saline (PBS) tablets	Calbiochem	524650	
Filter System 50mL with 0.22um pore	Corning	430320	
Centrifuge conical tube 50 mL	VWR	89039-658	
Single Flow Meter	Billups-Rothenberg	SMF3001	Measure gas flow in oxygen purge.
Hypoxia Incubator Chamber	StemCell	27310	Generates a hypoxic environment for the cell culture.
Traceable Dissolved Oxygen Meter	VWR	21800-022	
95% N ₂ / 5% CO ₂ Gas Mixture	Linde		Purges the environment of oxygen.

primary astrocyte immunofluorescence - step 6			
Phosphate buffer saline (PBS) tablets	Calbiochem	524650	
Formaline Solution Neutral Buffer 10%	Sigma-Aldrich	HT501128	Solution used to fix cells.
Methanol	Fisher	A4544	Solution used to fix cells.
Non-ionic surfactant (Triton X-100)	Sigma-Aldrich	T8787	
Fetal bovine serum (FBS)	GibCo. Company	10437-010	Serum-supplement for the cell culture.
Anti-NeuN	Cell Signaling	24307	Detects mature neurons, serves to validate the astrocytic culture.
Anti-PCNA	Cell Signaling	2586	Detects proliferating cells.
Propidium Iodide (PI)	Sigma-Aldrich Company	P4170	Apoptosis staining.
Anti-Olig1	Abcam	AB68105	Detects mature oligodendrocytes.
Anti-Iba1+	Wako	016-20001	Detects microglial cells.
Anti-GFAP Conjugated with Cy3	Sigma-Aldrich Company	C9205	Detects reactive astrocytes in the treated cells.
Alexa Fluor 488	Molecular Probe Life Technology	A1101	Anti-Mouse Secondary Antibody.
Alexa Fluor 555	Molecular Probe Life Technology	A21428	Anti-Rabbit Secondary Antibody.
4',6'-diamidino-2-phenylindole (DAPI)	Sigma-Aldrich Company	D9542	Nuclear staining.
Confocal microscope	Olympus		