

Isolation and Functional Analysis of Arteriolar Endothelium of Mouse Brain Parenchyma

 Md A. Hakim¹, Paulo W. Pires², Erik J. Behringer¹
¹Department of Basic Sciences, Loma Linda University ²Departments of Physiology, Surgery and Neurosurgery and Sarver Heart Center, University of Arizona College of Medicine Tucson

Corresponding Author

 Erik J. Behringer
 ebehringer@llu.edu

Citation

 Hakim, M.A., Pires, P.W., Behringer, E.J. Isolation and Functional Analysis of Arteriolar Endothelium of Mouse Brain Parenchyma. *J. Vis. Exp.* (181), e63463, doi:10.3791/63463 (2022).

Date Published

March 11, 2022

DOI

10.3791/63463

URL

jove.com/video/63463

Materials

Name	Company	Catalog Number	Comments
Amplifiers	Molecular Devices, Sunnyvale, CA, USA	Axoclamp 2B & Axoclamp 900A	
Audible baseline monitors	Ampol US LLC, Sarasota, FL, USA	BM-A-TM	
Bath Chiller (Isotemp 500LCU)	ThermoFisher Scientific	13874647	
Borosilicate glass capillaries (Pinning)	Warner Instruments	G150T-6	
Borosilicate glass capillaries (Sharp Electrodes)	Warner Instruments	GC100F-10	
Borosilicate glass capillaries (Trituration)	World Precision Instruments (WPI), Sarasota, FL, USA	1B100-4	
BSA: Bovine Serum Albumin	Sigma	A7906	
CaCl ₂ : Calcium Chloride	Sigma	223506	
Collagenase (Type H Blend)	Sigma	C8051	
Cover Glass (2.4 × 5.0 cm)	ThermoFisher Scientific	12-548-5M	
Data Acquisition Digitizer	Molecular Devices, Sunnyvale, CA, USA	Digidata 1550A	
Dissection Dish (Glass Petri with Charcoal Sylgard bottom)	Living Systems Instrumentation, St. Albans City, VT, USA	DD-90-S-BLK	
Dithioerythritol	Sigma	D8255	
DMSO: Dimethyl Sulfoxide	Sigma	D8418	
Elastase (porcine pancreas)	Sigma	E7885	
Endoplasmic Reticulum Tracker (ER-Tracker Red, BODIPY TR Glibenclamide)	ThermoFisher Scientific	E34250	
Fiber optic light sources	Schott, Mainz, Germany & KL200, Zeiss	Fostec 8375	
Flow Control Valve	Warner Instruments	FR-50	
Fluorescence system interface, ARC lamp & power supply, hyperswitch and PMT	Molecular Devices, Sunnyvale, CA, USA	IonOptix Systems	
Forceps (Fine-tipped, sharpened)	FST	Dumont #5 & Dumont #55	

Function Generator	EZ Digital, Seoul, South Korea	FG-8002	
Fura-2 AM dye	Invitrogen, Carlsbad, CA, USA	F14185	
Glucose	Sigma-Aldrich (St. Louis, MO, USA)	G7021	
HCl: Hydrochloric Acid	ThermoFisher Scientific (Pittsburgh, PA, USA)	A466250	
Headstages	Molecular Devices	HS-2A & HS-9A	
HEPES: (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)	Sigma	H4034	
Inline Solution Heater	Warner Instruments	SH-27B	
KCl: Potassium Chloride	Sigma	P9541	
MgCl ₂ : Magnesium Chloride	Sigma	M2670	
Microforge	Narishige, East Meadow, NY, USA	MF-900	
Micromanipulator	Siskiyou	MX10	
Micropipette puller (digital)	Sutter Instruments, Novato, CA, USA	P-97 or P-1000	
Microscope (Nikon-inverted)	Nikon Instruments Inc, Melville, NY, USA	Ts2	
Microscope (Nikon-inverted)	Nikon Instruments Inc	Eclipse TS100	
Microscope objectives	Nikon Instruments Inc	20X (S-Fluor) and 40X (Plan Fluor)	
Microscope platform (anodized aluminum; diameter, 7.8 cm)	Warner Instruments	PM6 or PH6	
Microscope Stage (Aluminum)	Siskiyou, Grants Pass, OR, USA	8090P	
Microsyringe Pump Controller	World Precision Instruments (WPI), Sarasota, FL, USA	SYS-MICRO4	
MTA: 2-Methylthioadenosine diphosphate trisodium salt	Tocris	1624	
NaCl: Sodium Chloride	Sigma	S7653	
NaOH: Sodium Hydroxide	Sigma	S8045	
Nuclear Stain (NucBlue Live ReadyProbes Reagent; Hoechst 33342)	ThermoFisher Scientific	R37605	
Oscilloscope	Tektronix, Beaverton, Oregon, USA	TDS 2024B	
Papain	Sigma	P4762	
Phase contrast objectives	Nikon Instruments Inc	(Ph1 DL; 10X & 20X)	
Plasma Membrane Stain (CellMask Deep Red)	ThermoFisher Scientific	C10046	
Plexiglas superfusion chamber	Warner Instruments, Camden, CT, USA	RC-27	
Scissors (3 mm & 7 mm blades)	Fine Science Tools (or FST), Foster City, CA, USA	Moria MC52 & 15000-00	
Scissors (Vannas style; 9.5 mm & 3 mm blades)	World Precision Instruments	555640S, 14364	
Stereomicroscopes	Zeiss, NY, USA	Stemi 2000 & 2000-C	
Syringe filter (0.22 µm)	ThermoFisher Scientific	722-2520	
Temperature Controller (Dual Channel)	Warner Instruments	TC-344B or C	
Valve Control System	Warner Instruments	VC-6	
Vibration Isolation Table	Technical Manufacturing, Peabody, MA, USA	Micro-g	