

Use of the MicroSiM (μ SiM) Barrier Tissue Platform for Modeling the Blood-Brain Barrier

Molly C. McCloskey¹, Pelin Kasap², Michelle Trempel¹, Louis P. Widom³, Julia Kuebel¹, Kaihua Chen¹, Thomas R. Gaborski³, Britta Engelhardt², James L. McGrath¹

¹Department of Biomedical Engineering, University of Rochester ²Theodor Kocher Institute, University of Bern ³Department of Biomedical Engineering, Rochester Institute of Technology

Corresponding Author

James L. McGrath
jmcgrath@ur.rochester.edu

Citation

McCloskey, M.C., Kasap, P., Trempel, M., Widom, L.P., Kuebel, J., Chen, K., Gaborski, T.R., Engelhardt, B., McGrath, J.L. Use of the MicroSiM (μ SiM) Barrier Tissue Platform for Modeling the Blood-Brain Barrier. *J. Vis. Exp.* (2023), e65258, doi:10.3791/65258 (2024).

Date Published

January 12, 2024

DOI

10.3791/65258

URL

jove.com/video/65258

Materials

Name	Company	Catalog Number	Comments
Antibodies			
CD31 Polyclonal antibody	Thermo Fisher Scientific	PA5-32321	
Claudin-5 mouse IgG antibody, 4C3C2	Thermo Fisher Scientific	35-2500	
Goat α -Mouse IgG Alexa Fluor 488	Thermo Fisher Scientific	A11001	
Goat α -Rabbit IgG Alexa Fluor 568	Thermo Fisher Scientific	A11011	
Hoechst 33342	Life Technologies	H3570	
NG2, mouse IgG2a, 9.2.27	Millipore	MAB2029	
Occludin mouse IgG antibody, OC-3F10	Thermo Fisher Scientific	33-1500	
PDGFR β , rabbit IgG antibody, 28E1	Cell Signaling Technology	3169	
VE-cadherin mouse IgG2B Clone # 123413	R&D Systems	MAB9381	
ZO-1 rabbit IgG antibody, polyclonal	Thermo Fisher Scientific	40-2200	
Chemicals, peptides, and recombinant proteins			
100% Methanol	VWR	101443-718	Can be substituted with similar products
16% Paraformaldehyde	Thermo Fisher Scientific	28908	Can be substituted with similar products
Accutase	Thermo Fisher Scientific	A1110501	
Acetic acid	Sigma-Aldrich	695092	
B27 supplement	Thermo Fischer Scientific	17504044	
bFGF	R&D Systems	233-FB-025	
Collagen IV from human placenta	Sigma-Aldrich	C5533	
Collagen, Type I solution from rat tail	Sigma-Aldrich	C3867-1VL	Can be substituted with similar products

DMEM/Ham's F12	Thermo Fisher Scientific	11320033	
EGM-2 Endothelial Cell Growth Medium-2 BulletKit	Lonza	CC-3162	
Essential 6 medium	Thermo Fisher Scientific	A1516401	
Fetal bovine serum (FBS)	Peak Serum	PS-FB1	
Fibronectin from bovine plasma	Sigma-Aldrich	F1141	
Fibronectin human protein, plasma	ThermoFisher Scientific	33016015	Can be substituted with similar products
hESFM	Thermo Fisher Scientific	11111044	
Lucifer Yellow CH dilithium salt	Sigma-Aldrich	861502	
Normal goat serum	Thermo Fisher Scientific	500627	Can be substituted with similar products
PBS without calcium, magnesium	Cytiva	SH30256.01	Can be substituted with similar products
Pericyte Medium	ScienCell	1201	Use complete kit (includes supplements)
Poly-L-Lysine, 10 mg/mL	ScienCell	0413	
Triton X-100	JT Baker	X198-05	Can be substituted with similar products
UltraPure DNase/RNase-Free Distilled Water	Invitrogen™	10977015	Can be substituted with similar products
Experimental models: Cell lines			
Blood-brain barrier hCMEC/D3 cell line	Sigma-Aldrich	SCC066	
Human brain vascular pericytes	ScienCell	1200	
iPS(IMR90)-4 human induced pluripotent stem cells	WiCell	RRID:CVCL_C437	
Glassware and Plasticware			
150 mm TC-treated Cell Culture Dish with 20 mm Grid	Falcon	353025	
Clamps	VWR	MFLX06832-10	
Corning tissue culture plates (6-well)	Corning	3506	
Flat 96-well non-binding microplates	Greiner Bio-One	655900	
Microscope Slide Device Holder	SiMPore	USIM-SB	Dimensions compatible with microscope slide holders
Nunc 15 mL Conical Sterile Polypropylene Centrifuge Tubes	Thermo Fischer Scientific	339651	
Nunc 50 mL Conical Sterile Polypropylene Centrifuge Tubes	Thermo Fischer Scientific	339653	Tube caps can be filled with water for cell culture
P20-200 pipette tips	VWR	76322-516	Alternate brands may not seal as effectively into ports
Transwell filters (12 well, 12 mm, 0.4 µm pore size, PC)	CoStar	3401	
Instruments			
10X Objective (For Andor)	Nikon Instruments Inc.	MRD00105	Air; WD: 4 mm; NA 0.45
10X Objective (For Nikon)	Nikon Instruments Inc.	MRP40102	Air; WD: 6.2 mm; NA 0.25
40X Objective (LWD) (For Andor)	Nikon Instruments Inc.	MRD77410	Water immersion; WD: 590-610 mm; NA 1.15
Andor Spinning Disc Confocal microscope	Oxford Instruments, Andor		
Nikon Eclipse Ts2 Phase Contrast Microscope	Nikon Instruments Inc.		Can be substituted with similar products
TECAN Infinite M200	TECAN		Can be substituted with similar products

Other			
Advanced PAP Pen	Millipore Sigma	Z672548	2 mm tip is recommended
Assembly kit with fixtures	SiMPore	USIM-JIGSET	Including fixture A1, A2, B1, and B2
Camera Adapter for Microscopes	AmScope	CA-CAN-SLR Canon SLR / D-SLR	Φ23.2 - Φ30 adapter fits Nikon Eclipse Ts2
Canon EOS Rebel SL3 DSLR Camera	Canon	EOS 250D, BH #CAEDRSL3B, MFR #3453C001	Can be substituted with similar products
Cell strainer, 40 μm	Millipore Sigma (Corning)	CLS431750	
Component 1	SiMPore	USIM-C1	
Component 2	SiMPore	USIM-C2	
Membrane chips	SiMPore	NPSN100-1L	Other chips formats are available
SMD handling tweezer double angle	Techni-Tool	758TW003	"Chip Tweezers"
Stainless steel precision type GG tweezer	Techni-Tool	758TW534	"Straight Tweezers"
Software			
Fiji	ImageJ		For image processing
Fusion	Oxford Instruments, Andor		Instructions for version 2.3.0.44
i-control	TECAN		Instructions for version 2.0
Imaris	Oxford Instruments		For image processing. Instructions for version 9.9.0