

A Triple Culture Cell System Modeling the Human Blood-Brain Barrier

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Citation

Rizzi, E., Deligne, C., Dehouck, L., Bilardo, R., Sano, Y., Shimizu, F., Kanda, T., Resmini, M., Gosselet, F., Dehouck, M.P., Mysiorek, C. A Triple Culture Cell System Modeling the Human Blood-Brain Barrier. *J. Vis. Exp.* (177), e63134, doi:10.3791/63134 (2021).

Date Published

November 30, 2021

DOI

10.3791/63134

URL

jove.com/video/63134

Materials

Name	Company	Catalog Number	Comments
Cell Culture			
Astocyte Medium (AM)	ScienCell	1801	
Astrocyte Growth Supplement	ScienCell	1852	Astrocyte Growth Supplement is provided in the AM set.
Cell culture dish 100 mm	Corning	430293	100 mm x 20 mm; dish used for the thawing of ECs and PCs before the triculture setting
Cell culture dish 150 mm	Corning	430599	The height of these dishes (25 mm) allows the seeding of PCs in the reverted insert for the setting of the triculture model.
Collagen I	Corning	354236	Rat tail
Dulbecco's Modified Eagle Medium	Gibco	31600-083	Powder
Endothelial Cell Growth Supplement	ScienCell	1051	Endothelial Cell Growth Supplement is provided in the ECM set.
Endothelial Cell Medium (ECM)	ScienCell	1001	
Fetal Calf Serum	Sigma	F7524	
Gelatin	Sigma	G2500	2% gelatin from porcine skin in PBS-CMF
Gentamicin	BiochromA6	A-2712	
Glucose	Sigma	G6152	Powder
Glutamine	Merck	1002891000	
Human Brain Cortex Astrocytes	ScienCell	1800-SC	
Malassez cell counting chamber	vWR	HECH40453702	The count was performed manually.
Matrigel	Corning	354230	Extracellular matrix-based hydrogel
Penicillin/Streptomycin	ScienCell	0503	Penicillin/Streptomycin solution is provided in the ECM and AM sets.

Poly-L-lysine	ScienCell	0413	
Steritop	Millipore System	SCGPT0SRE	0.22 µm pore size
Transwell insert	Corning	3401	0.4 µm pore polycarbonate filter
Trypsin/EDTA neutralization solution	ScienCell	0113	
Trypsin/EDTA solution	ScienCell	0103	
Immunocytochemistry			
SEA BLOCK blocking buffer	ThermoScientific	37527	
Alexa Fluor 568 anti-Mouse secondary antibody	ThermoFisher	A11031	Dilution 1:500
Alexa Fluor 568 anti-Rabbit secondary antibody	ThermoFisher	A11036	Dilution 1:500
Anti-Claudin-5 primary antibody	InVitrogen	34-1600	Dilution 1:100
Anti-Desmin primary antibody	Abcam	ab6322	Dilution 1:200
Anti-Glial Fibrillary Acidic Protein primary antibody	Dako	Z0334	Dilution 1:500
Anti-Platelet-Derived Growth Factor-β primary antibody	Abcam	ab51090	Dilution 1:200
Anti-VE-cadherin primary antibody	Abcam	ab207732	Dilution 1:200
Anti-Zona Occludens-1 primary antibody	InVitrogen	61-7300	Dilution 1:200
Normal Goat Serum	Sigma	G6767	
ProLong Diamond Antifade Mountant with DAPI	Invitrogen	P36962	
Gene expression			
NucleoSpin Rna/Protein Macherey Nagel Kit	Macherey-Nagel	740,933,250	
96 multiplate well	Biorad	HSP9601	
iSCRIPT	Biorad	1708841	
Sealer sheet	Biorad	MSB1001	
SsoFast EvaGreen Supermix	Biorad	172-5201	
Protein expression			
2x Laemli Sample Buffer	Biorad	161-0737	Add 50 µL of bMercaptoetanol to 950 µL of Laemmli Buffer and store at -20°C. Dilute 1:1 with the protein sample for the assay.
Anti-Breast Cancer Resistance Protein primary antibody	Abcam	ab207732	Pre-treatment 15 minutes at RT under agitation; dilution 1:1000, O.N. at 4°C
Anti-Claudin-5 primary antibody	Abcam	ab15106	Pre-treatment 5 minutes at 95°C; dilution 1:1000, O.N. at 4°C
Anti-Glucose Transporter 1 primary antibody	Millipore	07-1401	Pre-treatment 5 minutes at 95°C; dilution 1:1000, O.N. at 4°C
Anti-Mouse secondary antibody	Dako	P0447	Dilution 1:5000 in TBS-Tween
Anti-P-glycoprotein primary antibody	Genetex	GTX23364	Pre-treatment 15 minutes at RT under agitation; dilution 1:400, 3 hours at RT
Anti-Rabbit secondary antibody	Dako	P0448	Dilution 1:8000 in TBS-Tween
Anti-Transferrin Receptor primary antibody	Abcam	ab84036	Pre-treatment 5 minutes at 95°C; dilution 1:1000, O.N. at 4°C
Anti-Zona occludens-1 primary antibody	Abcam	ab216880	Pre-treatment 5 minutes at 95°C; dilution 1:1000, O.N. at 4°C
Criterion TGX Gel	Biorad	5678083	
ECL Prime Solution	Amersham	RPN2236	Revelation solution to keep in the dark

Phosphatase inhibitor cocktail 2	Sigma	P5726	
Phosphatase inhibitor cocktail 3	Sigma	P0044	
Protease Inhibitor	Sigma	P8340	
Protein Standards	Biorad	161-0373	Molecular weight markers
RIPA 10x	Millipore	20-188	
TBS 10x	Biorad	1706435	
TRIS-Glycine	Biorad	1610771	
Tween	Biorad	1706531	
BBB integrity assay			
Sodium Fluorescein	Ampresco	0681	λ_{ex} = 490 nm; λ_{em} = 525 nm
Elacridar	Sigma	SML0486	GF120918
FITC-Dextran 20 kDa	Sigma	FD-20S	λ_{ex} = 490 nm; λ_{em} = 525 nm
Rhodamine 123	Sigma	R8004	λ_{ex} = 501 nm; λ_{em} = 538 nm
Synergy TM H1	BioTek Instruments		Fluorescent multiplate reader
Nanogel Transport			
Syringe	Terumo	SS+01T1	1 mL syringe
Filter	FisherScientific	15161499	0.2 μ m PTFE membrane filter, 15 mm diameter
N-Isopropylacrylamide (NIPAM)-based hydrogels			The nanogels (NGs) used in the study are provided by our collaborator in Queen Mary University London, Department of Chemistry. The NGs are covalently tagged with a fluorescent molecule (λ_{ex} = 477 nm; λ_{em} = 540 nm). NGs are freeze dried and shipped as powder, in this state they are stable at room temperature for long period of time.