

Materials List for:

# An *In Vitro* Model of the Blood-brain Barrier Using Impedance Spectroscopy: A Focus on T Cell-endothelial Cell Interaction

Ivan Kuzmanov<sup>1</sup>, Alexander M. Herrmann<sup>1</sup>, Hans-Joachim Galla<sup>2</sup>, Sven G. Meuth<sup>1</sup>, Heinz Wiendl<sup>1</sup>, Luisa Klotz<sup>\*1</sup>

<sup>1</sup>Department of Neurology, University Hospital Münster

<sup>2</sup>Institute of Biochemistry, University of Münster

\*These authors contributed equally

Correspondence to: Heinz Wiendl at [heinz.wiendl@ukmuenster.de](mailto:heinz.wiendl@ukmuenster.de), Luisa Klotz at [luisa.klotz@ukmuenster.de](mailto:luisa.klotz@ukmuenster.de)

URL: <https://www.jove.com/video/54592>

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## Materials

Name	Company	Catalog Number	Comments
cellZscope	nanoAnalytics GmbH	<a href="http://www.nanoanalytics.com">www.nanoanalytics.com</a>	including: 24-well Cell Module, Controller, PC with cellZscope software v2.2.2
Ultracentrifuge	Thermo Scientific	<a href="http://www.thermoscientific.com">www.thermoscientific.com</a>	SORVALL RC 6+; rotor F21S-8x50y; for MBMEC isolation
flow cytometer	Beckman Coulter	<a href="http://www.beckmancoulter.com">www.beckmancoulter.com</a>	for analysis of T cell transmigration
FlowJo7.6.5 software	Tree Star	<a href="http://www.flowjo.com">www.flowjo.com</a>	for analysis of T cell transmigration
Oak Ridge centrifuge tubes, PC	Thermo Fisher Scientific	3118-0050	50 ml; for MBMEC isolation
Transwell membrane inserts - pore size 0.4 µm	Corning	3470	for TEER measurement as the main readout
Transwell membrane inserts - pore size 3 µm	Corning	3472	for TEER measurement as the quality control prior to T-cell transmigration assay
24-well cell culture plate	Greiner	650 180	flat-bottom; for MBMEC culture
96-well cell culture plate	Costar	3526	round-bottom; for immune cell culture
QuadroMACS Separator	Miltenyi Biotec	130-090-976	for T cell and B cell isolation; supports MACS LS columns
OctoMACS Separator	Miltenyi Biotec	130-042-109	for dendritic cell isolation; supports MACS MS columns
Neubauer counting chamber	Marienfeld	MF-0640010	for cell counting
Cell strainer, 70 µm	Corning	352350	for immune cell isolation
Cell strainer, 40 µm	Corning	352340	for immune cell isolation
MACS MultiStand	Miltenyi Biotec	130-042-303	for immune cell isolation
MACS LS separation columns	Miltenyi Biotec	130-042-401	for T cell and B cell isolation
MACS MS separation columns	Miltenyi Biotec	130-042-201	for dendritic cell isolation
Mouse CD4 MicroBeads	Miltenyi Biotec	130-049-201	for CD4 <sup>+</sup> T cell isolation
Mouse CD19 MicroBeads	Miltenyi Biotec	130-052-201	for B cell isolation
Mouse CD11c MicroBeads	Miltenyi Biotec	130-052-001	for dendritic cell isolation
Collagen type IV from human placenta	Sigma	C5533	for MBMEC coating solution
Fibronectin from bovine plasma	Sigma	F1141-5MG	for MBMEC coating solution
Collagenase 2 (CSL2)	Worthington	LS004176	for MBMEC isolation
Collagenase/Dispase (C/D)	Roche	11097113001	for MBMEC isolation
DNase I	Sigma	DN25	for MBMEC isolation

Fetal Bovine Serum (FBS)	Sigma	F7524	for MBMEC isolation
Bovine Serum Albumin (BSA)	Amresco	0332-100G	for MBMEC isolation
Percoll	Sigma	P1644-1L	for MBMEC isolation
DMEM (+ GlutaMAX)	Gibco	31966-021	for MBMEC isolation and MBMEC culture medium
Penicillin/Streptomycin	Sigma	P4333	for MBMEC isolation and MBMEC culture medium
Phosphate-Buffered Saline (PBS)	Sigma	D8537	for MBMEC and immune cell isolation
Heparin	Sigma	H3393	for MBMEC culture medium
Human Basic Fibroblast Growth Factor (bFGF)	PeptoTech	100-18B	for MBMEC culture medium
Puromycin	Sigma	P8833	for MBMEC culture medium; only for the first three days
0.05% Trypsin-EDTA	Gibco	25300-054	for harvesting MBMECs
Collagenase Type IA	Sigma	C9891	for dendritic cell isolation
Trypan Blue solution, 0.4%	Thermo Fisher Scientific	15250061	for cell counting
EDTA	Sigma	E5134	for immune cell isolation
IMDM + 1% L-Glutamin	Gibco	21980-032	for T cell culture medium
X-VIVO 15	Lonza	BE04-418Q	protect from light; for B cell culture medium
$\beta$ -mercaptoethanol	Gibco	31350-010	for B cell culture medium
L-Glutamine (100x Glutamax)	Gibco	35050-061	for B cell culture medium
mouse MOG <sub>35–55</sub> peptide	Biotrend	BP0328	for antigen-specific T cell activation
purified anti-mouse CD3 Ab	BioLegend	100302	clone 145-2C11; for polyclonal T cell activation
purified NA/LE anti-mouse CD28 Ab	BD Pharmingen	553294	clone 37.51; for polyclonal T cell activation
Recombinant Murine IFN- $\gamma$	PeptoTech	315-05	for T-cell transmigration assays
Recombinant Murine TNF- $\alpha$	PeptoTech	315-01A	for T-cell transmigration assays
NA/LE purified anti-mouse IFN- $\gamma$ antibody	BD Biosciences	554408	clone XMG1.2; recommended final concentration: 20 $\mu$ g/ml
Granzyme B Inhibitor II	Calbiochem	368055	recommended final concentration: 10 $\mu$ M
PE anti-mouse CD4 antibody	Biolegend	116005	clone RM4-4; for analysis of T cell transmigration