

Materials List for:

# Generation and Culture of Blood Outgrowth Endothelial Cells from Human Peripheral Blood

Mark L. Ormiston<sup>1</sup>, Mark R. Toshner<sup>2</sup>, Fedir N. Kiskin<sup>1</sup>, Christopher J. Z. Huang<sup>1</sup>, Emily Groves<sup>1</sup>, Nicholas W. Morrell<sup>1</sup>, Amer A. Rana<sup>1</sup>

<sup>1</sup>Department of Medicine, University of Cambridge

<sup>2</sup>Papworth Hospital

Correspondence to: Mark L. Ormiston at [mlo32@medschl.cam.ac.uk](mailto:mlo32@medschl.cam.ac.uk), Amer A. Rana at [ar332@cam.ac.uk](mailto:ar332@cam.ac.uk)

URL: <https://www.jove.com/video/53384>

DOI: [doi:10.3791/53384](https://doi.org/10.3791/53384)

## Materials

Name	Company	Catalog Number	Comments
<b>For blood collection</b>			
60 ml syringe with luer-lok tip	BD	309653	
19G Surflo Winged Infusion Set	Terumo	SV-19BL	
50 ml conical centrifuge tube	StarLab	E1450	2 per donor
Sodium Citrate	Martindale Pharmaceuticals	270541	
<b>Name</b>			
<b>Company</b>			
<b>Catalog Number</b>			
<b>Comments</b>			
<b>For buffy coat isolation</b>			
Ficoll-Paque Plus	GE Healthcare	17-1440-03	
Dulbecco's PBS (without Ca <sup>2+</sup> and Mg <sup>2+</sup> )	Sigma-Aldrich	D8537	
Sterile wrapped plastic transfer pipettes	Appleton Woods	KC231	
Turk's Solution	Millipore	1.093E+09	
<b>Name</b>			
<b>Company</b>			
<b>Catalog Number</b>			
<b>Comments</b>			
<b>For cell culture, passaging and freezing cells</b>			
Type 1 Collagen (derived from rat tail)	BD Biosciences	35-4236	
Dulbecco's PBS (without Ca <sup>2+</sup> and Mg <sup>2+</sup> )	Sigma-Aldrich	D8537	
0.02M Acetic Acid	Sigma-Aldrich	A6283	prepared in reagent grade water
Endothelial Growth Medium-2MV (containing Bullet Kit, but not serum)	Lonza	CC-3202	<b>Note:</b> It is essential that the medium does not contain heparin. <b>Do not use</b> EGM-2.
Fetal Bovine Serum (U.S.), Defined	Hyclone	SH30070	
10x Trypsin EDTA	Gibco	T4174	Dilute to 1x in PBS prior to use
Heat Inactivated FBS	Gibco	10500-064	
DMEM	Gibco	41965-039	
DMSO	Sigma-Aldrich	276855	
Nalgene Mr. Frosty Freezing Container	Sigma-Aldrich	C1562	
<b>Name</b>			
<b>Company</b>			
<b>Catalog Number</b>			
<b>Comments</b>			
<b>For flow cytometric characterization</b>			
FITC-conjugated mouse anti-human CD14	BD Biosciences	555397	Mouse IgG <sub>1</sub> k, Clone: WM59 Dilution: 1:20

FITC-conjugated mouse anti-human CD31	BD Biosciences	555445	Mouse IgG <sub>1</sub> k, Clone: WM59 Dilution: 1:20
APC-conjugated mouse anti-human CD34	BD Biosciences	555824	Mouse IgG <sub>1</sub> k, Clone: 581/CD34 Dilution: 1:20
FITC-conjugated mouse anti-human CD45	BD Biosciences	560976	Mouse IgG <sub>1</sub> k, Clone: HI30 Dilution: 1:20
APC-conjugated mouse anti-human VEGFR2	R&D Systems	FAB357A	Mouse IgG <sub>1</sub> , Clone: 89106 Dilution: 1:10
FITC-conjugated mouse IgG <sub>1</sub> k isotype control	BD Biosciences	555748	Clone: MOPC-21 Dilution: 1:20
APC-conjugated mouse IgG <sub>1</sub> k isotype control	BD Biosciences	555751	Clone: MOPC-21 Dilution: 1:20
APC-conjugated mouse IgG <sub>1</sub> k isotype control	R&D Systems	IC002A Dilution: 1:10	Clone: 11711
EDTA, 0.5M solution	Sigma-Aldrich	E7889	
<b>Name</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments</b>
<b>For immunofluorescent microscopy</b>			
Corning Costar 24-well tissue culture plate	Sigma-Aldrich	CLS3527	
Paraformaldehyde	Sigma-Aldrich	158127	
BSA	Sigma-Aldrich	A7906	
Polysorbate 20	Sigma-Aldrich	P2287	
Monoclonal mouse anti-human CD34 antibody	R&D Systems	MAB72271	Clone 756510, IgG <sub>1</sub> , use at 10 µg/ml
Polyclonal goat anti-human VE-cadherin (CD144)	R&D Systems	AF938	Antigen affinity- purified IgG, use at 1:300
Monoclonal rabbit anti-human Von Willebrand Factor (vWF)	Abcam	ab154193	Clone EPSISR15, use at 1:250
Donkey anti-mouse IgG (H+L) secondary antibody, Alexa Fluor 488 conjugate	Life Technologies	A-21202	Polyclonal, 2 mg/ml, use at 1:200
Donkey anti-goat IgG (H+L) secondary antibody, Alexa Fluor 488 conjugate	Life Technologies	A-11055	Polyclonal, 2 mg/ml, 1:200
Donkey anti-rabbit IgG (H+L) secondary antibody, Alexa Fluor 568 conjugate	Life Technologies	A-10042	Polyclonal, 2 mg/ml, 1:200
DAPI (4',6-Diamidino-2-phenylindole dihydrochloride)	Sigma-Aldrich	D9542	use at 1 µg/ml