

Materials List for

In vitro Three-Dimensional Sprouting Assay of Angiogenesis using Mouse Embryonic Stem Cells for Vascular Disease Modeling and Drug Testing

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Materials

Name	Company	Catalog Number	Comments
2-mercaptoethanol	Milipore, Merck	805740	Biohazard: adequate safety instructions should be taken when handling
Agar Noble	Difco, BD Pharmigen	214220	
Alexa Fluor 555 goat anti rat IgG	Life technologies	A21434	
APC conjugated rat anti-mouse PECAM-1 antibody (clone MEC13.3)	BD Biosciences	551262	
APC Rat IgG2a κ Isotype Control (Clone R35-95)	BD Biosciences	553932	
Axiovert 25 inverted phase contrast tissue culture microscope	ZEISS		
Basic Fibroblast Growth Factor-2 (bFGF)	Peprotech	450-33	
Benchtop Centrifuge, Allegra X-15R	Beckman Coulter	392932	
Biosafety cabinet BioVanguard (Green Line)	Telstar	133H401001	
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A9418	
Cell counting chamber, Buerker, 0.100mm	Marienfeld	640211	
Cell culture dishes 60 x 15mm	Corning	353802	
Cell culture dishes, 35 x 10 mm	Corning	353801	
Cell culture plates 12-well	Corning	3512	
CFX96 Touch Real-Time PCR Detection System	Biorad	1855196	
Chicken serum	Sigma-Aldrich	C5405	
CHIR-99021 (CT99021) HCl	Selleckchem	S2924	
Collagen I, High Concentration, Rat Tail, 100mg	Corning	354249	

Collagenase A	Roche	10103586001	
Confocal Laser Scanning Microscope, TCS SP5	Leica		
Cover glasses, 24 × 50 mm	Vwr	631-0146	
DAPT γ secretase inhibitor	Sigma Aldrich	D5942	
DC101 anti mouse VEGFR-2 Clone	BioXcell	BP0060	
DC101 isotype rat IgG1	BioXcell	BP0290	
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	D2438-5X	Biohazard: adequate safety instructions should be taken when handling
DPBS (10x), no calcium, no magnesium	Gibco, Thermofisher scientific	14200067	
EDTA 40 mM	Gibco, Thermofisher scientific	15575-038	
Embryonic stem-cell Fetal Bovine Serum	Gibco, Thermofisher scientific	16141-079	Should be lot-tested for maximum ES cell viability and growth. Heat inactivate at 60°C and store at -20 °C for up to 1 year
Eppendorf Microcentrifuge 5415R	Eppendorf AG	Z605212	
Erythropoietin, human (hEPO), 250 U (2.5 μ g) (1 mL)	Roche	11120166001	
ESGRO Recombinant Mouse LIF Protein (10 ⁷ units 1 mL)	Milipore, Merck	ESG1107	
Falcon tubes 15 mL	Greiner Bio-One	188271	
Falcon tubes 50 mL	Greiner Bio-One	227270	
Filter tip ,clear ,sterile F.Gilson, P-200	Greiner Bio-One	739288	
Filter tip ,clear ,sterile F.Gilson, P10	Greiner Bio-One	771288	
Filter tip ,clear ,sterile F.Gilson, P1000	Greiner Bio-One	740288	
FITC conjugated anti- α Smooth Muscle Actin (SMA) (clone 1A4)	Sigma Aldrich	F3777	
FITC conjugated rat anti-mouse CD45 (clone 30-F11)	Biologend	103107	
FITC Rat IgG2b, κ Isotype Ctrl Antibody (clone RTK4530)	Biologend	400605	
Fluorescent mounting media	DAKO	S3023	
Gascompress	Cutisoft	45846	
Gauze Cutisoft 10 x 10 cm	Bsn Medical	45844_00	
Gel blotting paper, Grade GB003	Whatman	WHA10547922	
Gelatin solution, type B	Sigma-Aldrich	G1393-100 ml	
Glasgow's MEM (GMEM)	Gibco, Thermofisher scientific	21710082	
IHC Zinc Fixative	BD Pharmigen	550523	
IncuSafe CO2 Incubator	PHCBI	MCO-170AICUV-PE	
Interleukin-6, human (hIL-6)	Roche	11138600001	
L-Glutamine 200 mM	Gibco, Thermofisher scientific	25030-024	
MEM Non-Essential Amino Acids Solution (100x)	Gibco, Thermofisher scientific	11140035	
Microscope slide box	Kartell Labware	278	
Microscope slide, Starfrost	Knittel glass	VS113711FKB.0	
Mm_Cdh5_1_SG QuantiTect Primer Assay	Qiagen	QT00110467	
Mm_Eng_1_SG QuantiTect Primer Assay	Qiagen	QT00148981	

Mm_Epha4_1_SG QuantiTect Primer Assay	Qiagen	QT00093576	
Mm_Ephb2_1_SG QuantiTect Primer Assay	Qiagen	QT00154014	
Mm_Flt1_1_SG QuantiTect Primer Assay	Qiagen	QT00096292	
Mm_Flt4_1_SG QuantiTect Primer Assay	Qiagen	QT00099064	
Mm_Gapdh_3_SG QuantiTect Primer Assay	Qiagen	QT01658692	
Mm_Kdr_1_SG QuantiTect Primer Assay	Qiagen	QT00097020	
Mm_Notch1_1_SG QuantiTect Primer	Qiagen	QT00156982	
Mm_Nr2f2_1_SG QuantiTect Primer Assay	Qiagen	QT00153104	
Mm_Pecam1_1_SG QuantiTect Primer	Qiagen	QT01052044	
Mm_Tek_1_SG QuantiTect Primer Assay	Qiagen	QT00114576	
Mouse (ICR) Inactivated Embryonic Fibroblasts (2 M)	Gibco, ThermoFisher scientific	A24903	Store vials in liquid nitrogen (195.79 °C) indefinitely
Mouse embryonic stem cell line 7AC5/EYFP (ATCC SCRC-1033)	ATCC	SCRC-1033	Generated by Dr A Nagy, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Ave, Toronto, Ontario, M5G 1X5, Canada. [Hadjantonakis, A. K., et al. Mechanisms of Development. 76 (1–2), 79–90 (1998)].
Mouse embryonic stem cell lines Acvrl1 +/- and Acvrl1 +/+			Generated at Leiden University Medical Centre [Thalgott, J.H. et al. Circulation. 138 (23), 2698–2712 (2018)].
Mouse embryonic stem cells line E14			Provided by M Letarte laboratory and generated according to Cho, S. K., et al. Blood. 98 (13), 3635–3642 (2001).
Mouse embryonic stem cells line R1 (ATCC SCRC-1011)	ATCC	SCRC-1011	Generated by Dr A Nagy, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Ave, Toronto, Ontario, M5G 1X5, Canada. [Nagy, A., et al. Proceedings of the National Academy of Sciences of the United States of America. 90 (18), 8424–8428 (1993)].
Mouse embryonic stem cells line Z/Red (strain 129/Ola)			Generated by Dr A Nagy, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, 600 University Ave, Toronto, Ontario, M5G 1X5, Canada [Vintersten, K., et al. Genesis. 40 (4), 241–246 (2004)].
NanoDrop 1000 UV/VIS Spectrophotometer	Thermo Fischer Scientific	ND-1000	
PD0325901	Selleckchem	S1036	
PDGF-BB, Recombinant Human	Peprtech	100-14B	
Pecam-1 antibody, Rat Anti-Mouse	BD Biosciences	550274	
Penicillin-streptomycin (10,000 U/mL)	Gibco, ThermoFisher scientific	15140122	
Petri dish, PS, 94/16 mm, standard ,with vents, sterile	Greiner Bio-One	633181	
Pipetboy acu 2	Integra-Biosciences	155 019	
Pipetman G Multichannel P8 x 200G	Gilson	F144072	

Pipetman G Starter Kit, 4 Pipette Kit, P2G, P20G, P200G, P1000G	Gilson	F167360	
Recombinant Human BMP-4 Protein	R&D Systems	314-BP	
RNeasy Plus mini Kit	QIAGEN	74134	
Serological pipettes, 10 mL	Greiner Bio-One	607 180	
Serological pipettes, 25 mL	Greiner Bio-One	760 180	
Serological pipettes, 5 mL	Greiner Bio-One	606 180	
Sodium hydroxide (NaOH)	Merck	106498	
Sodium pyruvate 100 mM	Gibco, Thermofisher scientific	11360039	
Test tubes 5ml round-bottom with cell-strainer cap	Corning	352235	
Thermal cycler, T100	Biorad	1861096	
Triton X-100 (BioXtra)	Sigma Aldrich	T9284	
Trypan Blue Solution, 0.4%	Gibco, Thermofisher scientific	15250061	
Trypsin (2.5%)	Gibco, Thermofisher scientific	15090046	
Vacuum Filter/Storage Bottle System, 500 mL	Corning	430758	
VEGFA ¹⁶⁵ , recombinant murine	Peprtech	450-32	
Water, Sterile	Fresenius-Kabi	B230531	
Waterbath, Lab-Line Digital	Thermo Fischer Scientific	18052A	