

Materials List for:

Bioengineering of Humanized Bone Marrow Microenvironments in Mouse and Their Visualization by Live Imaging

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Materials

Name	Company	Catalog Number	Comments
Ficoll-Paque	Ge Healthcare	17-1440-03	
Cd34 positive selection Kit	Stemcell	18056	Store at 4 °C.
Magnet	Stemcell	18000	
Anti-human CD3 antibody, clone OKT-3	Bioxcell	BE0001-2	Store at 4 °C. Used for T cell depletion in primary AML samples.
Cytokines (IL3, G-CSF, and TPO)	PeproTech	200-03, 300-23 and 300-18	For doing the stock: Dilute each one of the cytokines in 100 µL of water and mix them. Add 200 µL, do aliquots of 45 µL and Store at -20 °C.
DMSO	Sigma	D4540	
FBS	Life technologies	10270106	Heat-inactivate at 56 °C during 30 min, do aliquots and freeze down. Warm in 37 °C water bath before use.
MEM-α	Invitrogen	32571-028	Store at 4 °C. Warm in 37 °C water bath before use.
Myelocult H5100	corning	5100	Store at 4 °C. Warm in 37 °C water bath before use.
199	Gibco	41150-020	Store at 4 °C. Warm in 37 °C water bath before use.
hMSC-FBS, Heat-inactivated	Gibco	12662-029	Store at -20 °C. Warm in 37 °C water bath before use.
P/S	Sigma-Aldrich	P0781	Store at -20 °C. Warm in 37 °C water bath before use.
ECGS	Millipore	02-102	Dilute in culture media and use 0.22 mm filter.
HEPES	Sigma-Aldrich	S1558-50ML	Store at 4 °C.
Heparin	Sigma-Aldrich	H3149	Store at 4 °C.
Glutamine	Gibco	25030	Store at -20 °C.
Collagen 1 coated cell culture plate	corning	354505	
Trypsin-EDTA solution	Thermo-Fisher	25200056	Store at -20 °C. Warm in 37 °C water bath before use.
hMSC	Lonza	PT-2501	Alternatively, hMSCs we also kindly provided by Dr. Dosquet (University Paris Diderot, Paris) from human bone marrow obtained during orthopaedic surgery under ethical approval 10-038 from IRB00006477.

VeraVec HUVEC endothelial cells	Angiocrine bioscience	hVera101	Alternatively, other human endothelial cell source may be used.
Human hematopoietic cells			Umbilical Cord blood or primary Acute Myeloid Leukemia (AML) samples were obtained from the Royal London Hospital (London, UK) after informed consent and protocol of use was approved by the East London Ethical Committee and carried out in accordance with the Declaration of Helsinki.
Gelfoam, Size 12 - 7mm	Pfizer	00009-0315-08	Alternative supplier: Febelco
PBS	Thermo-Fisher	10010023	
Surgical material	Multiple		Sterile forceps, tweezers and sharp scissors.
Sterile tissues			Heat-sterilize paper tissues.
1 mL Syringe with needle of 25G	Terumo	SS+01H25161	
Ultra Low Attachment Multiple Well Plates	Corning	3473 or 3471	
BMP2	Noricon	rhBMP-2	Dilute at 5 mg/mL in acetic acid 50 mM and store at 4 °C.
Thrombin	Sigma	T8885	Dilute in CaCl ₂ 2%, 500 mL per vial, and store at 4 °C.
Fibrinogen	Sigma	F3879	Dilute at 4 mg/100 mL in PBS. Store at -20 °C. Warm before use.
Tissue-culture dishes 35 mm x 10 mm	Falcon	353001	
U-Botton 96 well plate	Falcon	353077	
NSG mice	The Jackson Laboratory	5557	NSG mice were a kind gift from Dr Leonard Shultz (The Jackson Laboratory).
Chlorhexidine	G9		Dilute 1:10 before use
Carprofen	Pfizer	Rimadyl	5 mg/kg of mouse
Buprenorphine	Alstoe	Vetergesic	0.1 mg/kg of mouse body weight
Isoflurane	Abbott	B506	Induction of anaesthesia 2%, maintenance 1%
Trimmer	Wella	Contura HS61	
Surgical glue	3M	Vetbond	
carbomer (polyacrylic acid) as Ophthalmic gel	Novartis	Viscotears Liquid Gel	
Human Normal Immunoglobulin	Gammaflex	10g vial	100 mL/mouse intravenously, 30 min before infusion of specific antibody.
NT-QTracker	Invitrogen	Q21021MP	Vessel-pooling agent. Administrate 15 mL/mouse intravenously 5 min before imaging.
AF488-hCD45	Biolegend	304017	100 mL/mouse intravenously, 30 min before imaging.
FITC-hCD31	BD Pharmigen	555445	100 mL/mouse intravenously, 30 min before imaging.
Super glue	Loctite	Super Glue	
Micro-Drill Kit	IDEAL - Fisher Scientific	NC9010016	
Microsurgical microscope			No specific brand/company is advised.

LSM 710 NLO	Zeiss		Upright confocal microscope with motorized stage, two-photon laser and 20X 1.0 NA water immersion lens. Alternatively, a microscope with similar specifications could be used.
MaiTai "High Performance" fully automated 1-box 517 mode-locked Ti:Sapphire laser with DeepSee dispersion compensation	Spectra-Physics		
Formalin solution, neutral buffered, 10%	Sigma-Aldrich	HT501128	
Ethanol	Sigma-Aldrich	32294	
Osteosoft	Millipore	1.01728.1000	
Polysine slices	Thermo scientific	J2800AMNZ	
Antigen unmasking solution	Vector	H-3300	Store at 4 °C. Dilute 1:100 in H ₂ O for working solution.
Triton 100x	Sigma-Aldrich	T9284	
Bovine serum albumin (BSA)	Sigma-Aldrich	A96470	Store at 4 °C.
Normal Goat serum (NGS)	Sigma-Aldrich	G9023	Store at 4 °C.
Endomucin Antibody	Santa Cruz	sc-65495	Store at 4 °C.
hVimentin antibody	Santa Cruz	sc-6260	Store at 4 °C.
hCD31 antibody	DAKO	M0823	Store at 4 °C.
hCD45 antibody	DAKO	M0701	Store at 4 °C.
ADRP (Perilipin2)	antibodies-online	ABIN283918	Store at 4 °C.
Goat anti mouse secondary antibodies	Invitrogen	A11029 or A11005	Store at 4 °C.
Goat anti Rabbit secondary antibodies	Invitrogen	A11037 or A11008	Store at 4 °C.
Goat anti Rat secondary antibodies	Invitrogen	A11007 or A21247	Store at 4 °C.
Sudan Black	Sigma	S2380	Prepare a stock of 1% sudan black in ethanol 70%. Store at RT. Prepare working solution of 0.1% sudan black in ethanol 70% and filter using filter-paper before use.
DAPI	Sigma	D8417	Prepare stock in H ₂ O at 100 mg/mg. Store at 4 °C.
Fluorescent mounting media	Dako	S3023	Add DAPI before use (1:400 from DAPI stock).
Cover glass	VWR	631-0147	