

Materials List for:

Isolation and Culture Expansion of Tumor-specific Endothelial Cells

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Materials

Name	Company	Catalog Number	Comments
Antibiotic-Antimycotic	Sigma-Aldrich	A5955	
Dulbecco's Modified Eagle's medium (1 g/L D-glucose) (LG-DMEM)	Gibco	11885-084	
EGM-2 Bullet Kit	Lonza	CC4176	Not all components used
Fetal bovine serum (Hyclone)	Thermo Scientific	SH30071.03	Heat inactivated at 56 °C for 30 min
Nu-Serum IV	Corning	CB-51004	
Hank's Balanced Salt Solution (HBBS)	Gibco	14175-095	
Phosphate-buffered saline (PBS)	Gibco	14190-144	
FACS buffer			0.5 % BSA and 2 mM EDTA in PBS, filtered through a 0.22 µm filter
75% v/v ethanol for disinfection			
Anti-PE microbeads	Miltenyi Biotec	130-048-801	
Bovine serum albumin (BSA) fraction V, 7.5%	Gibco	15260-37	
Cell freezing media (Bambanker)	Wako Chemicals	302-14681	
Collagenase type II	Worthington Biochemical	LS004176	Make stock concentration 2 mg/ml in HBSS
Deoxyribonuclease I (DNase)	Worthington Biochemical	LS002004	Make stock concentration 1 mg/ml in PBS
Dil-Ac-LDL	Biomedical Technologies	BT-902	
EDTA, 0.5M, pH 8.0	Cellgro	46-034-CL	
Enzymatic cell detachment solution (Accutase)	Sigma-Aldrich	A6964-100ML	
Gelatin, 2% in water, tissue culture grade	Sigma-Aldrich	G1393-100ML	Dilute in PBS to make 0.5% gelatin solution
Mouse FcR Blocking Reagent	Miltenyi Biotec	130-092-575	
Neutral protease (Dispase)	Worthington Biochemical	LS02104	Make stock concentration 2.5 U/ml in HBSS
PE-rat anti-mouse CD31 antibody	BD Pharmingen	553373	
RBC lysis buffer (BD Pharm Lyse)	BD Pharmingen	555899	
Sterile water			
Trypan blue, 0.4 %	Life Technologies	15250-061	
10 mm tissue culture dishes	Corning		

15 ml conical tubes (sterile)	Corning		
50 ml conical tubes (sterile)	Corning		
6-well tissue culture plates	Corning		
Tissue-dissociator tubes (gentleMACS) C tubes)	Miltenyi Biotech	130-093-237	
Cell Separator (MidiMACS)	Miltenyi Biotech	130-042-302	
Cell strainer 100 µm	Corning	352360	
Cloning rings (assorted sizes)	Bel-Art Products	378470000	
Cryotubes	Thermo Scientific		
Dissecting board			Sterilize or disinfect with 75% v/v ethanol before use
Dissecting forceps and scissors			Sterilize before use
Dissecting pins 2"			Sterilize before use
FACS tubes with 35 µm filter cap	Corning	352235	
Filter cup (Stericup, 0.22 µm)	Millipore	SCGPU05RE	
Fine-tip marker			
Hemocytometer			
LS Columns	Miltenyi Biotech	130-042-401	
Magnetic Multistand	Miltenyi Biotech	130-042-303	
Tissue adhesive (Vetbond)	3M	1469SB	
Centrifuge	Eppendorf	5810R	Or a centrifuge with similar capacity for 15 ml and 50 ml conical tube centrifugation
Tissue culture hood			
Tissue dissociator (gentleMACS)	Miltenyi Biotech	130-093-235	Preset program "m_impTumor_01" used for tissue dissociation
Liquid nitrogen freezer			
Microplate or rotary shaker			
Phase contrast light microscope			