

Materials List for:

Pan-Myeloid Differentiation of Human Cord Blood Derived CD34⁺ Hematopoietic Stem and Progenitor Cells

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Materials

Name	Company	Catalog Number	Comments
0.4% Trypan blue solution	Thermo Fisher Scientific	15250-061	Dilute working stock to 0.2% in sterile 1x PBS
0.5 M UltraPure Ethylene diamine tetra acetic acid, pH 8.0	Gibco	15575-038	
10x Hanks Balanced Salt Solution (HBSS)	Invitrogen	14185052	Dilute to 1x with sterile distilled water & pH to 7.2
2.5% Trypsin, no phenol red	Thermo Fisher Scientific	15090046	Dilute working stock to 1x with sterile 1x PBS
30 µm Pre-separation filters	Miltenyi biotech	130-041-407	
35% sterile Bovine serum albumin	Sigma-Aldrich	A7979	
7-AAD	Biolegend	420404	Used as a live/dead stain to eliminate dead cells from FACS analysis
Anti-human CD10-FITC antibody (Clone HI10a)	Biolegend	312207	Use 1:20 dilution
Anti-human CD11b-FITC (activated) antibody (Clone CBRM1/5)	Biolegend	301403	Use 1:5 dilution
Anti-human CD123-APC antibody (Clone 6H6)	Biolegend	306012	Use 1:20 dilution
Anti-human CD14-PE antibody (Clone M5E2)	Biolegend	301806	Use 1:20 dilution
Anti-human CD19-FITC antibody (Clone 4G7)	BD Biosciences	347543	Use 1:5 dilution
Anti-human CD235a-APC antibody (Clone GA-R2 (HIR2))	BD Biosciences	551336	Use 1:20 dilution
Anti-human CD235a-FITC antibody (Clone HIR2)	Biolegend	306609	Use 1:50 dilution
Anti-human CD34-APC-Cy7 antibody (Clone 581)	Biolegend	343514	Use 1:20 dilution
Anti-human CD38-PE antibody (Clone HIT2)	Biolegend	303506	Use 1:20 dilution
Anti-human CD3-FITC antibody (Clone UCHT1)	Biolegend	300405	Use 1:20 dilution
Anti-human CD41a-PerCP-Cy5.5 antibody (Clone HIP8)	Biolegend	303720	Use 1:20 dilution
Anti-human CD45Ra-PE-Cy7 antibody (Clone HI100)	Biolegend	304126	Use 1:20 dilution
Anti-human CD66b-PE-Cy7 antibody (Clone G10F5)	Biolegend	305116	Use 1:20 dilution

Anti-human CD7-FITC antibody (Clone CD7-6B7)	Biolegend	343103	Use 1:20 dilution
Dimethyl sulfoxide (DMSO)	Fisher Scientific	BP231-100	Filter sterilize before use
Dulbecco's Modified Eagle Medium (DMEM) powder with L-Glutamine	Gibco	12100046	Reconstitute 1 packet to make 1 L of DMEM media with sodium bicarbonate, 10% FBS & 1% penicillin & streptomycin
Fetal bovine serum, Australian source, heat inactivated	Omega Scientific	FB-22 Lot #609716	
Human CD34 microbead kit	Miltenyi biotech	130-046-702	
Human Thrombopoietin (TPO), research grade	Miltenyi biotech	130-094-011	Make a stock of 100 µg/mL in 1x PBS + 0.1% BSA. Use 50 ng/mL for both myeloid differentiation & stimulation medium
L-Glutamine	Omega Scientific	GS-60	2 mM concentration in stimulation medium
LS Columns	Miltenyi biotech	130-042-401	
MACS Multi stand	Miltenyi biotech	130-042-303	
MidiMACS magnetic separator	Miltenyi biotech	130-042-302	
MNC fractionation media (Ficol-Paque PLUS)	GE Healthcare Biosciences	17-1440-03	
MS-5 cells			Gift from the laboratory of Gay Crooks, UCLA
Paraformaldehyde	Sigma-Aldrich	P6148	Heat 800 mL of 1x PBS in a glass beaker on a stir plate in a chemical hood to ~65 °C. Add 10 g of paraformaldehyde powder. To completely dissolve the paraformaldehyde, raise the pH by adding 1 N NaOH. Cool and filter the solution and make up the volume to 1 L with 1x PBS. Adjust the pH to 7.2.
Penicillin & Streptomycin	Sigma-Aldrich	P4458-100ml	
Poly-L lysine	Sigma-Aldrich	P2636	Make a 10 mg/mL stock in 1x PBS
Recombinant human erythropoietin-alpha (rHu EPO-α)	BioBasic	RC213-15	Make a stock of 2000 units/mL in 1x PBS + 0.1% BSA. Use 4 units/mL for myeloid differentiation
Recombinant human fibronectin fragment (RetroNectin)	Takara	T100B	Use 20 µg/mL diluted in sterile 1x PBS to coat wells prior to stimulation of CD34+ HSCs.
Recombinant human Flt-3 ligand (rHu Flt-3L)	BioBasic	RC214-16	Make a stock of 100 µg/mL in 1x PBS + 0.1% BSA. Use 5 ng/mL for myeloid differentiation & 50 ng/mL in stimulation medium
Recombinant human interleukin-3 (rHu IL-3)	BioBasic	RC212-14	Make a stock of 100 µg/mL in 1x PBS + 0.1% BSA. Use 5 ng/mL for myeloid differentiation & 20 ng/mL in stimulation medium
Recombinant human stem cell factor (rHu SCF)	BioBasic	RC213-12	Make a stock of 100 µg/mL in 1x PBS + 0.1% BSA. Use 5 ng/mL for myeloid differentiation & 50 ng/mL in stimulation medium
Serum free medium (X-Vivo-15)	Lonza	04-418Q	
Sodium bicarbonate	Fisher Scientific	BP328-500	
Wright-Giemsa stain, modified	Sigma-Aldrich	WG16-500	Use according to manufacturer's instructions

Equipment			
BD LSR II flow cytometer	BD Biosciences		
Centrifuge	Sorvall Legend RT		
Light microscope	Olympus		