

Materials List for:

Megakaryocyte Differentiation and Platelet Formation from Human Cord Blood-derived CD34⁺ Cells

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Materials

Name	Company	Catalog Number	Comments
Cell Culture Reagents			
Recombinant Human TPO	Miltenyi Biotec	130-094-013	
StemSpan SFEM II	Stem Cell Technologies	9605	Serum-free media for CD34+ cells
Name	Company	Catalog Number	Comments
CD34 Isolation Reagents			
CD34 MicroBead kit ultrapure	Miltenyi Biotec	130-100-453	This kit includes the FcR human IgG blocking reagent and CD34 microbeads. These beads contain the anti-CD34 antibody clone QBEND/10. Use a different anti-CD34 clone for purity check (e.g. clone 8G12).
Lymphoprep	Alere Technologies	1114545	Lymphocyte separation media (density 1.077 g/mL)
Sterile separation buffer (SB)	Miltenyi Biotec	130-091-221	This buffer contains phosphate buffered saline (PBS), pH 7.2 containing 0.5% bovine serum albumin and 2 mM EDTA. It can be prepared using sterile, cell culture grade components. De-gas before use because air bubbles can block the column.
Name	Company	Catalog Number	Comments
Flow Cytometry and Cell Staining Reagents			
PE Mouse anti-Human CD34	BD Biosciences	340669	Clone 8G12. This can be used for CD34 purity check. Final antibody concentration 1:10 dilution.
PerCP mouse anti-human CD45	BD Biosciences	347464	1:10 dilution
PerCP isotype control	BD Biosciences	349044	1:10 dilution
FITC Mouse anti-Human CD41a	BD Biosciences	340929	Final antibody concentration 1:5 dilution.
APC Mouse anti-Human CD42b	BD Biosciences	551061	This antibody can also be used to detect mature MK (the percentage of positive cells is usually lower than with anti CD42a). Final antibody concentration 1:10 dilution.
Alexa Fluor 647 Mouse anti-Human CD42a	AbD Serotec	MCA1227A647T	Currently distributed by Bio-Rad. Final antibody concentration 1:10 dilution.

Alexa Fluor 647 Mouse Negative Control	AbD Serotec	MCA928A647	Currently distributed by Bio-Rad. Isotype control antibody
Anti von Willebrand factor rabbit polyclonal	Abcam	AB6994	1:200 dilution
V450 mouse anti-humna CD41a	BD Biosciences	58425	1: 20 dilution
V450 isotype control	BD Biosciences	580373	1:20 dilution
PAC1-FITC antibody	BD Biosciences	340507	1:10 dilution
Anti CD62p mouse monoclonal	Abcam	AB6632	1:200 dilution
Alexa Fluor 488 goat anti rabbit IgG	Invitrogen	A11008	1:100 dilution
Alexa Fluor 594 goat anti mouse IgG	Invitrogen	A11020	1:100 dilution
Ig Isotype Control cocktail-C	BD Biosciences	558659	Isotype control antibody
Propidium iodide	Sigma Aldrich	P4864	
CountBright Absolute Counting Beads	Molecular Probes, Invitrogen	C36950	Counting beads
Name	Company	Catalog Number	Comments
Materials			
LS columns	Miltenyi Biotec	130-042-401	Smaller and larger columns are also commercially available
MidiMACS Separator magnet	Miltenyi Biotec	130-042-302	
MACS MultiStand	Miltenyi Biotec	130-042-303	
Falcon 5mL round bottom polypropylene FACS tubes, with Snap Cap, Sterile	In Vitro technologies	352063	
Glass slides	Menzel-Glaser	J3800AMNZ	
Mounting media with DAPI	Vector Laboratories	H-1200	Antifade mounting medium with DAPI
Name	Company	Catalog Number	Comments
Equipment			
Inverted microscope	Leica	DMIRB inverted microscope	
Fluorescent microscope	Zeiss	Vert.A1	
Cell analyser	BD Biosciences	FACS Canto II	
Cytospin centrifuge	ThermoScientific	Cytospin 4	
Name	Company	Catalog Number	Comments
Software			
Cell analyser software	BD Biosciences	FACS Diva Software	
Single cell analysis software	Tree Star	FlowJo	
Fluorescent microscope software	Zeiss	Zen 2 blue edition	