

Immunophenotyping and Cell Sorting of Human MKs from Human Primary Sources or Differentiated *In Vitro* from Hematopoietic Progenitors

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Citation

Acebes-Huerta, A., Martínez-Botía, P., Martín Martín, C., Bernardo, Á., Rodríguez, A.R., Vicente-Ayuso, M.C., Benavente Cuesta, C., Gutiérrez, L. Immunophenotyping and Cell Sorting of Human MKs from Human Primary Sources or Differentiated *In Vitro* from Hematopoietic Progenitors. *J. Vis. Exp.* (174), e62569, doi:10.3791/62569 (2021).

Date Published

August 7, 2021

DOI

10.3791/62569

URL

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Materials

Name	Company	Catalog Number	Comments
130 micron Nozzle	BD	643943	required for MK sorting
5810R Centrifuge	Eppendorf		Cell isolation and washes
A-4-62 Swing Bucket Rotor	Eppendorf		Cell isolation and washes
Aerospray Pro Hematology Slide Stainer / Cyto centrifuge	ELITech Group		Automatized cytology devise, where slides are stained with Mat-Grünwald Giemsa
CO ₂ Incubator Galaxy 170 S	Eppendorf		Cell Incubation
Cytospin 4 Cyto centrifuge	Thermo Scientific		To prepare cytopins
FACSAria IIu sorter	BD		Lasers 488-nm and 633-nm
FACSCanto II flow cytometer	BD		Lasers 488-nm , 633-nm and 405-nm
Olympus Microscope BX 41	Olympus		Microphotographs
Olympus Microscope BX 61	Olympus		Microphotographs
Zoe Fluorescent Cell Imager	BioRad		Microphotographs
To obtain PBMCs			
Lipids Cholesterol Rich from adult bovine serum	Sigma-Aldrich	L4646	or similar
Lymphoprep	Stem Cell Technologies	#07801	or similar
Penicillin-Streptomycin	Sigma-Aldrich	P4333	or similar
Recombinant human Erythropoietin (EPO)	R&D Systems	287-TC-500	or similar
Recombinant human stem cell factor (SCF)	Thermo Fisher Scientific, Gibco™	PHC2115	or similar

Recombinant human thrombopoietin (TPO)	Thermo Fisher Scientific, Gibco™	PHC9514	or TPO receptor agonists
StemSpan SFEM	Stem Cell Technologies	#09650	
Flow Cytometry Analyses			
Bovine Serum Albumin	Merck	A7906-100G	or similar
BD CompBead Anti-Mouse Ig, κ/ Negative Control Compensation Particles Set	BD	552843	Antibodies for human cells are generally from mouse.
BD Cytotfix/Cytoperm	BD	554714	or similar
BD FACS Accudrop Beads	BD	345249	
CD31 AF-647	BD	561654	Mouse anti-human
CD31 FITC	Immunostep	31F-100T	
CD34 FITC	BD	555821	Mouse anti-human
CD41 PE	BD	555467	Mouse anti-human
CD41 PerCP-Cy5.5	BD	333148	Mouse anti-human
CD42A APC	Immunostep	42AA-100T	We observed unspecific binding... that needs to be assessed
CD42A PE	BD	558819	Mouse anti-human
CD42B PerCP	Biolegend	303910	Mouse anti-human
CD49B PE	BD	555669	Mouse anti-human
CD61 FITC	BD	555753	Mouse anti-human
CD71 APC-Cy7	Biolegend	334109	Mouse anti-human
Hoechst 33342	Thermo Fisher Scientific	H3570	
Human BD Fc Block	BD	564219	Fc blocking - control
KIT PE-Cy7	Biolegend	313212	Mouse anti-human
Lineage Cocktail 2 FITC	BD	643397	Mouse anti-human
RNAse	Merck	R6513	or similar
Triton X-500	Merck	93443-500ML	or similar
Cell strainers for sorting			
CellTrics Filters 100 micrometers	Sysmex	04-004-2328	Cell strainers
Note: we do not specify general reagents/chemicals (PBS, EDTA, etc) or disposables (tubes, etc), or reagents specified in previous published and standard protocols - unless otherwise specified.			