

Materials List for:

Direct Induction of Hemogenic Endothelium and Blood by Overexpression of Transcription Factors in Human Pluripotent Stem Cells

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Materials

Name	Company	Catalog Number	Comments
Table 1. Induction of hPSCs differentiation with transcription factors and analysis of hemogenic endothelium and blood cells.			
pSIN4-EF1a-ETV2-IRES-Puro	Addgene	Plasmid #61061	Lentiviral Vector
pSIN4-EF1a-GATA2-IRES-Puro	Addgene	Plasmid #61063	Lentiviral Vector
pSIN4-EF1a-GATA1-IRES-Puro	Addgene	Plasmid #61062	Lentiviral Vector
pSIN4-EF1a-TAL1-IRES-Puro	Addgene	Plasmid #61062	Lentiviral Vector
pSIN4-EF1a-LMO2-IRES-Puro	Addgene	Plasmid #61064	Lentiviral Vector
Hexadimethrine bromide (Polybrene)	Sigma-Aldrich	107689-10G	Cationic polymer used to increase the efficiency of infection
Y-27632 (Dihydrochloride) ROCK inhibitor	STEMCELL Technologies	72302	RHO/ROCK pathway inhibitor Inhibits ROCK
StemPro Accutase Cell Dissociation Reagent	Life Technologies	A11105-01	Cell Dissociation Reagent
Incomplete (growth factor- free) culture medium mTeSR1 Custom formulation	WiCell Research Institute (Madison, WI)	MCF	Serum-free mTeSR1 medium without bFGF and TGFb
human SCF	Peprotech	300-07	Premium grade
human TPO	Peprotech	300-18	Research grade
human FGF-basic	Peprotech	100-18B	Premium grade
CD144 (VE-cad) FITC	BD Biosciences	560411	Endothelial marker (FACS)
CD226 PE	BD Biosciences	338305	Hematopoietic (FACS)
CD43 PE	BD Biosciences	560199	Hematopoietic (FACS)
CD73 APC	R&D Systems	FAB5795A	Endothelial marker (FACS)
CD45 APC	BD Biosciences	555485	Hematopoietic (FACS)
7AAD	Life Technologies	A1310	Live/Dead assay (FACS)
Paraformaldehyde	Sigma-Aldrich	P6148-500G	Cell fixation
Triton X-100	Sigma-Aldrich	T9284-500ML	Permeabilization
FBS	Fisher Scientific	SH3007003	Fetal bovine serum
Mouse anti-human CD43	BD Biosciences	551457	Pure, primary antibody for Immunofluorescence (IF) staining
Rabbit anti-human VE-cadherin	BenderMedSystem	BMS158	Primary (IF)
Anti-rabbit Alexa Fluor 488-conjugated	JacksonResearch	715-486-152	Secondary (IF)
Anti-mouse Alexa Fluor 594-conjugated	JacksonResearch	715-516-150	Secondary (IF)

DAPI nucleic acid stain	Life Technologies	D1306	Live/Dead assay (IF)
Clonogenic medium MethoCult H4435 Enriched	STEMCELL Technologies	4435	CFC-assay
Wright Stain solution	Sigma -Aldrich	32857	Staining cytopins

Table 2. hPSCs culture.

Human Pluripotent Stem Cells (hPSCs)	WiCell Research Institute (Madison, WI)	hESCs (WA01, WA09) human Embryonic Stem cells; iPSCs (DF-19-9-7T, DF-4-3-7T) transgene-free induced Pluripotent Stem Cells	hPSCs are able to self-renew and to differentiate into cells of three germ layers
Complete serum-free medium for culture of hPSCs mTeSR1 media	WiCell Research Institute (Madison, WI)	M500	Serum-free medium with growth factors for feeder free culture of ESC/iPSCs
Matrigel	BD Biosciences/ Corning	356234	Matrix for maintenance of human ESC/iPSCs
DMEM/F-12, powder	Life Technologies	12500-062	Basal Medium
HyClone Dulbecco's PBS powder	Fisher Scientific	dSH30013.04	PBS
Dispase II, powder	Life Technologies	17105-041	Neutral protease, Cell dissociation

Table 3. Primers for detection of virus genomic integration.

Primer's Name	Forward (Fwd) 5' -->3'	Reverse (Rev) 5'-->3'	Discription
pSIN EF1a Fwd	TTC CAT TTC AGG TGT CGT GA	--	EF1a promoter sequence
GATA1 Rev	--	TCC CTG TAG TAG GCC AGT GC	Coding Region
GATA2 Rev	--	GGT TGG CAT AGT AGG GGT TG	Coding Region
TAL1 Rev	--	AGG CGG AGG ATC TCA TTC TT	Coding Region
LMO2 Rev	--	GGC CCA GTT TGT AGT AGA GGC	Coding Region
ETV2 Rev	--	GAA CTT CTG GGT GCA GTA AC	Coding Region