

Materials List for:

Development, Expansion, and *In vivo* Monitoring of Human NK Cells from Human Embryonic Stem Cells (hESCs) and Induced Pluripotent Stem Cells (iPSCs)

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Materials

Name	Company	Catalog Number	Comments
Materials			
Media			
BPEL media for Spin EB generation			
Iscove's Modified Dulbecco's Medium (IMDM)	Fisher Scientific	SH3022801	
F-12 Nutrient Mixture w/ Glutamax I	Invitrogen	31765035	
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A3311	Commercially available BSA can be cytotoxic to ES cells. Deionizing the solution can reduce the potential for cytotoxicity.
Poly(vinyl alcohol)	Sigma-Aldrich	P8136	
Linoleic Acid	Sigma-Aldrich	L1012	
Linolenic Acid	Sigma-Aldrich	L2376	
Synthechol 500X solution	Sigma-Aldrich	S5442	
α-monothioglycerol (α-MTG)	Sigma-Aldrich	S5442	
Protein-free hybridoma mix II	Invitrogen	12040077	
Ascorbic Acid 2-phosphate	Sigma-Aldrich	A8960	
Glutamax I	Invitrogen	35050061	
Insulin, Transferrin, Selenium 100X solution (ITS)	Invitrogen	41400-045	
Pen-Strep	Invitrogen	15140122	
NK Differentiation Media			
Dubecco's Modified Eagle Medium (DMEM)	Invitrogen	11965-118	
F-12 Media	Invitrogen	CX30315	
15% Human AB serum	Valley Biomed	HP1022HI	
5 ng/ml Sodium Selenite	Sigma-Aldrich	S5261	
50 uM ethanolamine	Sigma-Aldrich	E9508	
20 ng/ml ascorbic acid	Sigma-Aldrich	A8960	
25 uM 2-mercapt-ethanol (BME)	Gibco	21985	
2 mM L-glutamine	Gibco	CX30310	
1% Pen-Strep	Invitrogen	15140122	

Cytokines			
(all cytokines used fresh from frozen aliquots)			
SCF	PeproTech, Inc.	300-07	40 ng/ml in BPEL media; 20 ng/ml in NK medium). As noted by the Elefanty protocol, and as we discovered with a bad lot of BMP4, there can be lot differences with cytokines in regards to hematopoietic differentiation. Especially if buying cytokines in bulk, obtain a sample of the lot to test prior to purchase. Compare head-to-head with old lot.
rhBMP-4	R & D Systems	314-BP	20 ng/ml in BPEL media
rhVEGF	R&D Systems	293-VE	20 ng/ml in BPEL media
IL-2	PeproTech, Inc.	200-02	1 x 10 ⁵ U/ml given to mice after NK cell injection; 50 U/ml used in NK cell expansion protocol
IL-15	PeproTech, Inc.	200-15	10 ng/ml given to mice after NK cell injection (first 7 days only)
IL-3	PeproTech, Inc.	200-03	5 ng/ml in NK media
IL-7	PeproTech, Inc.	200-07	20 ng/ml in NK media
Flt-3-Ligand	PeproTech, Inc.	300-19	10 ng/ml in NK media
In vivo Imaging			
D-Luciferin Sodium Salt	Gold BioTechnology	Lucna-500	
TurboFP650 plasmid	Evrogen, Moscow, Russia	FP731	Subcloned into a Sleeping Beauty transposon based plasmid driven by the mCAGs promoter. Cells were then sorted on their expression of turboFP650 by FACS. Cells and plasmids can be obtained from our lab.
Equipment			
IVIS Spectrum Imaging System	Caliper Life Sciences		
Cells			
Membrane bound IL-21 expressing artificial antigen presenting cells	 MD Anderson, Houston, TX		contact: Dean A. Lee. http://www.jove.com/video/2540/expansion-purification-functional-assessment-human-peripheral-blood
Firefly luciferase expressing hESCs	University of Minnesota, Minneapolis, MN		contact: Dan S. Kaufman . H9 cells modified with a Sleeping Beauty transposon based method (references 13 and 14). Expression of firefly luciferase is driven by the mCAGGS promoter. Following the firefly luciferase gene is an IRES element at the 5' end of a GFP:zeocin fusion construct.
TurboFP650 expressing K562 cells	University of Minnesota, Minneapolis, MN		contact: Dan S. Kaufman. Description under plasmid comments section
Materials Table.			