

Materials List for

Experimental Melanoma Immunotherapy Model using Tumor Vaccination with a Hematopoietic Cytokine

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jove.com/video/64082

Materials

Name	Company	Catalog Number	Comments
0.25% trypsin-EDTA	Gibco	25200-056	
10% heat-inactivated FBS	Omega Scientific	FB-02	Lot# 209018
30G needle	BD Biosciences	305106	
96 well V-shape-bottom plate	SARSTEDT	83.3926.500	
B16 cell line expressing Fms-like tyrosine kinase 3 ligand (B16-FIT3L)	Gift of Dr. Stephen Schoenberger, LJI		FIT3L cDNAs were cloned into the pMG-Lyt2 retroviral vector, as in reference 5, Supplemental Figure 1
B16-F10 cell lines	ATCC	CRL-6475	
Centrifuge 5810R	Eppendorf		
Cytofix fixation buffer	BD Biosciences	BDB554655	Cell fixation buffer (4.2% PFA)
Cytofix/Cytoperm kit	BD Biosciences	554714	Fixation/Permeabilization Solution Kit
DNase I	Sigma	11284932001	
Dulbecco's Modified Eagle Medium (DMEM)	Corning	10013CV	
Electronic digital caliper	Fisherbrand	14-648-17	
FlowJo software	Tree Star		Flow cytometer data analysis
GolgiStop (protein transport inhibitor)	BD Biosciences	554724	1:1500 dilution
HEPES (1M)	Gibco	15630-080	
Ionomycin	Sigma	I0634	
Iscove's modified Dulbecco's medium (IMDM)	Thermo Fisher	12440053	
LSR-II cytometers	BD Biosciences		Flow cytometer
MEM nonessential amino acids	Gibco	11140-050	
penicillin and streptomycin	Gibco	15140-122	
Percoll	GE Healthcare Life Sciences	GE17-0891-02	density gradient specific medium
PMA	Sigma	P1585	
Red Blood Cell Lysing Buffer Hybri-Max liquid	Sigma	R7757-100ML	
RPMI 1640 medium	Corning	10-040-CV	

RS2000 X-ray Irradiator	Rad Source Technologies		
sodium pyruvate	Gibco	11360-070	
Sterile cell strainer 40 µm	Fisherbrand	22-363-547	
Sterile cell strainer 70 µm	Fisherbrand	22-363-548	
TL Liberase	Roche	477530	
Zombie Aqua fixable viability kit	BioLegend	423101	
Antibodies			
Anti-mCD45	BioLegend	103135	Clone: 30-F11 Fluorophore: BV570 Dilution: 1:200
Anti-mCD3ε	BioLegend	100327	Clone: 145-2C11 Fluorophore: PerCP-Cy5.5 Dilution: 1:200
Anti-mCD8	BioLegend	100730 100724	Clone: 53-6.7 Fluorophore: Alexa Fluor 700, Alexa Fluor 647 Dilution: 1:200
Anti-mCD4	BioLegend	100414	Clone: GK1.5 Fluorophore: APC-Cy7 Dilution: 1:200
Anti-mFoxp3	Thermo Fisher Scientific	11577382	Clone: FJK-16s Fluorophore: FITC Dilution: 1:100
Anti-m/hGzmb	BioLegend	372208	Clone: QA16A02 Fluorophore: PE Dilution: 1:100
Anti-mIFNγ	BioLegend	505826	Clone: XMG1.2 Fluorophore: PE-Cy7 Dilution: 1:100
Anti-mCD19	BioLegend	115543	Clone: 6D5 Fluorophore: BV785 Dilution: 1:100
Anti-mGr1	BioLegend	108423	Clone: RB6-8C5 Fluorophore: APC/Cy7 Dilution: 1:200
Anti-mCD11b	BioLegend	101223	Clone: M1/70 Fluorophore: Pacific blue Dilution: 1:100
Anti-mF4/80	BioLegend	123114	Clone: BM8 Fluorophore: PECy7 Dilution: 1:100
Anti-mCD11c	BioLegend	117328	Clone: N418 Fluorophore: PerCP Cy5.5 Dilution: 1:100
Anti-mMHCII	BioLegend	107622	Clone: M5/114.15.2 Fluorophore: AF700 Dilution: 1:400
Anti-mCD103	BioLegend	121410	Clone: 2E7 Fluorophore: Alexa Fluor 647 Dilution: 1:200
Anti-mCD86	BioLegend	105007	Clone: GL-1 Fluorophore: PE Dilution: 1:200
FC-blocker (Rat anti-mouse CD16/CD32)	BD Biosciences	553141	Clone: 2.4G2 Dilution: 1:200