

Materials List for:

Transduction and Expansion of Primary T Cells in Nine Days with Maintenance of Central Memory Phenotype

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Materials

Name	Company	Catalog Number	Comments
Gammaretrovirus preparation			
0.025% Trypsin, 0.01% EDTA	Gibco	R-001-100	
293T cells	ATCC	CRL-3216	
6 well plates, treated	CytoOne	CC7682-7506	
DMEM	Gibco	10569-010	
Heat-inactivated FBS	Hyclone	Sh30088.03	
Lipofectamine	Invitrogen	11668019	transfection reagent
Opti-Mem	Invitrogen	31985070	reduced serum media
pBS-CMV-gagpol	Addgene	35614	A gift from Dr. Patrick Salmon
pMSGV1 containing CAR P2A CXCR5	custom order from GenScript		
RD114			A gift from Dr. Ed Berger
T75 flasks	CytoOne	CC7682-4875	
VSV-G		pMD.G	A gift from Dr. Scott McIvor
T cell stimulation			
6 well plates, untreated	CytoOne	CC7672-7506	
Anti-CD28	NHP Reagent Resource	Clone: CD28.2	
Anti-macaque CD3	NHP Reagent Resource	Clone: FN18	
Phosphate buffered saline	Gibco	14190-144	
Rhesus macaque PBMC or CD8 T cells	WNPRC		Primary cells
For Fibronectin coating			
6 well plates, untreated	CytoOne	CC7672-7506	
BSA (Fraction V)	HyClone	SH 30574.02	
RetroNectin (1 mg/ml)	TaKaRa	T100A	
For T cell Expansion			
G-Rex 6 Well Plate	Wilson Wolf	P/N 80240M	Plates with gas permeable wells
Media Components			
b mercaptoethanol	Gibco	21985-023	
Heat-inactivated FBS	Hyclone	Sh30088.03	
IL-2	NCI Preclinical Repository		
Penicillin/Streptomycin/Glutamine	Gibco	10378-016	
X-Vivo-15 medium	Lonza	04-418Q	
Variations of Media used			

Basic medium:	X-Vivo 15 medium, 10% heat-inactivated FBS, 1 x Penicillin/Streptomycin/L-Glutamine		
Expansion medium:	Growth medium + 50 mM b mercaptoethanol		
Growth medium:	Basic medium + 50 IU/ml IL-2		Completion of media by addition of anti-CD28, IL-2 or b-mercaptoethanol should occur on the day of use.
Cell counting			
Countess cell counting chambers	Invitrogen	AMQAF1000	
Countess II FL Automated Cell Counter	Invitrogen	T10282	
Trypan blue, 0.4% solution	Invitrogen	T10282	
Flow Cytometry			
Alexa Fluor 647 Antibody Labeling Kit	Invitrogen	A20186	for conjugation of MBL antibody
anti-CD28-BV605	BD Biosciences	562976	
anti-CD3-AF700	BD Biosciences	557917	
anti-CD4-FITC	BD Biosciences	556615	
anti-CD8-BV788	BD Biosciences	563824	
anti-CD95-PerCP Cy5.5	BD Biosciences	561655	
anti-CXCR5-PE	eBioscience	12-1985-42	
anti-MBL	Invitrogen	MA1-40145-S6	
Flow Analysis software	FlowJo, LLC	FlowJo v10	
Flow Cytometer	Beckman	CytoFlex	
Live/Dead Near IR	Invitrogen	L10119	
Other equipment			
Aerosolve canisters to contain aerosol leakage	Beckman	SX4750	Safety equipment
Beckman Allegra Centrifuge	Beckman	Sterilgard e3	
Cell culture incubator	Thermo Fisher	Everlast 247	
Class II Laminar flow hood	Baker	Heracell Vios 160i	
Extra-Safe Disposable lab coat	Fisher Scientific	359232	Personal protective equipment
Microplate carriers with biocertified covers	Beckman	SX4750A	Safety equipment
Rocking platform	Benchmark	C10228	
Swinging bucket rotor	Beckman	X13-R	
X-Gen Nitrile gloves	Genesee		Personal protective equipment