

Materials List for:

Generating *De Novo* Antigen-specific Human T Cell Receptors by Retroviral Transduction of Centric Hemichain

Tingxi Guo^{1,2}, Toshiki Ochi^{*2}, Munehide Nakatsugawa^{*2}, Yuki Kagoya², Mark Anczurowski^{1,2}, Chung-Hsi Wang^{1,2}, Muhammed A. Rahman², Kayoko Saso², Marcus O. Butler^{1,2}, Naoto Hirano^{1,2}

¹Department of Immunology, University of Toronto

²Princess Margaret Cancer Centre, University Health Network

* These authors contributed equally

Correspondence to: Naoto Hirano at naoto.hirano@utoronto.ca

URL: <https://www.jove.com/video/54697>

DOI: [doi:10.3791/54697](https://doi.org/10.3791/54697)

Materials

Name	Company	Catalog Number	Comments
0.05% Trypsin-EDTA	Wisent Bioproducts	325-043-CL	
293GPG cells	Generated by Ory <i>et al.</i> (ref 8)		
Agar	Wisent Bioproducts	800-010-CG	
Agarose	Wisent Bioproducts	800-015-CG	
Ampicillin sodium salt	Wisent Bioproducts	400-110-IG	
Chloroform	BioShop	CCL402	
Deoxynucleotide (dNTP) Solution Mix	New England Biolabs	N0447L	
DMEM, high glucose, pyruvate	Life Technologies	11995065	
EcoRI	New England Biolabs	R0101S	
EZ-10 Spin Column DNA Gel Extraction Kit	BS353		
Fetal Bovine Serum	Life Technologies	12483020	
Ficoll-Paque Plus	GE Healthcare	17-1440-02	
Filter	Corning	431220	0.45 mm pore SFCA membrane
FITC-conjugated anti-human CD271 (NGFR) mAb	Biolegend	345104	clone ME20.4
FITC-conjugated anti-human CD3 mAb	Biolegend	300440	clone UCHT1
Gentamicin	Life Technologies	15750078	
Gibson Assembly Master Mix	New England Biolabs	E2611L	used for multi-piece DNA assembly
HLA-A2 pentamer	Proimmune	depends on antigenic peptide	HLA-A2/MART1 multimer used here was purchased from Proimmune
HLA/CD1d monomers	NIH Tetramer Core Facility		multimerize monomers according to protocol provided by NIH tetramer core facility
Human AB serum	Valley Biomedical	HP1022	
human CD3 microbeads	Miltenyi Biotec	130-050-101	
IOtest Beta Mark TCR V beta Repertoire Kit	Beckman Coulter	IM3497	
Jurkat 76 cells	Generated by Heemskerk <i>et al.</i> (ref 10)		
LB Broth	Wisent Bioproducts	800-060-LG	
LS MACS column	Miltenyi Biotec	130-042-401	

NEB 5-alpha Competent <i>E. coli</i>	New England Biolabs	C2987I	
NEBuffer 3.1	New England Biolabs	B7203S	used for EcoRI and NotI digestion
NotI	New England Biolabs	R0189S	
NucleoBond Xtra Midi	Macherey-Nagel	740410	used for plasmid purification
PC5-conjugated anti-human CD8 mAb	Beckman Coulter	B21205	clone B9.11
PG13 cells	ATCC	CRL-10686	
Phusion HF Buffer Pack	New England Biolabs	B0518S	
Phusion High-Fidelity DNA Polymerase	New England Biolabs	M0530L	
pMX retroviral vector	Cell Biolabs	RTV-010	
polybrene	Sigma-Aldrich	H-9268	
Proleukin (recombinant human interleukin-2)	Novartis	by Rx only	equivalent product can be purchased from Sigma-Aldrich
Purified anti-human CD3 antibody	Biolegend	317301	clone OKT3, used for T cell stimulation
RPMI 1640	Life Technologies	11875119	
SA-PE	Life Technologies	S866	used for multimerizing monomers from NIH tetramer core facility
SMARTer RACE 5'/3' Kit	Clontech	634858	
Sterile water	Wisent Bioproducts	809-115-LL	
SuperScript III First-Strand Synthesis System	Invitrogen	18080051	for cDNA synthesis
Syringe	BD	301604	10 ml, slip tip
Tetracycline hydrochloride	Sigma-Aldrich	T7660	
TransIT-293	Mirus Bio	MIR 2700	used to transfect 293GPG cells
TRIzol Reagent	Life Technologies	15596026	