

Materials List for:

# High-Efficiency Generation of Antigen-Specific Primary Mouse Cytotoxic T Cells for Functional Testing in an Autoimmune Diabetes Model

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## Materials

Name	Company	Catalog Number	Comments
2-Mercaptoethanol (50mM)	Gibco	21985-023	50 uM
5' RACE PCR	Clontech	634859	
anti-mouse CD28 antibodies	eBioscience	14-0281-86	final concentration at 1µg/ml
anti-mouse CD3e antibody	eBioscience	145-2C11	final concentration at 1µg/ml
Biotin Rat Anti-Mouse IFN-γ	BD Biosciences	554410	Working concentration at 0.5 µg/ml
BSA	Sigma	A7030	
Endo-free Maxi-Prep kit	Qiagen	12362	
Gentamicin	Gibco	15750-060	Final 50 µg/ml.
Heat inactivated FCS	Hyclone	SH30087.03	Final 10% FCS
HEPES (100X)	Gibco	15630-080	1X
IgG7-CLIP tetramer-BV421	NIH tetramer Facility at Emory	per approval	Working concentration at 6 µg/ml
IgG7-insulin P8E tetramer-BV421	NIH tetramer Facility at Emory	per approval	Working concentration at 6 µg/ml
Insulin-Transferrin-Selenium-Ethanolamine (ITS 100x)	ThermoFisher	51500056	Final concentraion is 1x
Lipofectamine 2000	Invitrogen	11668019	
LS Columns	Miltenyi Biotec	130-042-401	
MACS Separation Buffer	Miltenyi Biotec	130-091-221	
Mouse CD8a+ T Cell Isolation Kit	Miltenyi Biotec Inc	130-104-075	
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Opti-MEM medium	ThermoFisher	31985070	
Penicillin-Streptomycin (5000U/ml)	ThermoFisher	15070063	50 U/ml
Phoenix-ECO cells	ATCC	CRL-3214	
Phosphate-buffered saline (PBS)	Gibco	10010-023	
pMIG II	Addgene	52107	
pMSCV-IRES-GFP II	Addgene	52107	
Purified Rat Anti-Mouse IFN-γ	BD Biosciences	551216	Working concentration at 3 µg/ml
Red cell lysis buffer	Sigma	R7767	
RetroNectin	Takara	T100A	Working concentration at 50 µg/ml in PBS
rhIL-2 (stock concentration 10 <sup>5</sup> IU/ul)	Peptotech	200-02	Final concentration at 200 IU/ml
rmIL-7 ( stock concentration 50ng/ul)	R&D	407-ML-005	Final concentration at 0.5ng/ml

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RPMI-1640	Gibco	11875-093	
Sterile Cell Strainers	Fisher Scientific	22-363-548	
Tryple	Gibco	12605-028	