

Materials List for:

Retroviral Transduction of Helper T Cells as a Genetic Approach to Study Mechanisms Controlling their Differentiation and Function

Yogesh Singh^{1,2}, Oliver A. Garden³, Florian Lang², Bradley S. Cobb¹

¹Department of Comparative Biomedical Sciences, The Royal Veterinary College

²Institute of Physiology I, Cardiology & Vascular Medicine, Eberhard Karls University of Tuebingen

³Department of Clinical Science and Services, The Royal Veterinary College

Correspondence to: Bradley S. Cobb at bcobb@rvc.ac.uk

URL: <https://www.jove.com/video/54698>

DOI: [doi:10.3791/54698](https://doi.org/10.3791/54698)

Materials

Name	Company	Catalog Number	Comments
RPMI	Sigma	R8758	
DMEM	Sigma	D5671	
Penicillin Streptomycin solution	Sigma	P4333	
L-Glutamine	Sigma	G7513	
β-mercaptoethanol	Sigma	M3148	
DPBS	Sigma	D8537	
MIG vector	Addgene	Plasmid 9094	
pCL-Eco vector	Addgene	Plasmid 12371	
Cell strainer	BD Falcon	352350	
Magnetic beads mouse CD4 cell kit	Invitrogen (Dynabeads)	11415D	
Streptavidin Beads	Miltenyi Biotech	130-048-102	
MS cell separation columns	Miltenyi Biotech	130-042-201	
LS cell separation columns	Miltenyi Biotech	130-042-401	
CD25 Biotenylated MAb	BD Biosciences	85059	clone 7D4
CD62L Biotenylated MAb	BD Biosciences	553149	clone MEL-14
Polybrene (Hexadimethrine Bromide)	Sigma	107689	
Anti-CD3	eBiosciences	16-0031-85	clone 145-2C11
Anti-CD28	eBiosciences	16-0281-85	clone 37.51
Anti-IL-4	BD Biosciences	559062	clone 11B11
Anti-IFN-gamma	BD Biosciences	559065	clone XMG1.2
Anti-IL-2	BD Biosciences	554425	cloneJES6-5H4
Recombinant IL-12 p70	eBiosciences	14-8121	
Recombinant IL-4	BD Biosciences	550067	
Recombinant TGF-beta	eBiosciences	14-8342-62	
Recombinant IL-6	eBiosciences	14-8061	
Recombinant IL-2	eBiosciences	14-8021	
PMA	Sigma	P8139	
Ionomycin	Sigma	I0634	
Brefeldin A	eBiosciences	00-4506	
Paraformaldehyde	Sigma	16005	Paraformaldehyde is toxic so use appropriate caution when handling

Foxp3 staining buffer set	eBiosciences	00-5523	
Anti-CD4 FITC	eBiosciences	11-0041	clone GK1.5
Anti-CD8a perCP-cy5.5	eBiosciences	45-0081-80	clone 53-6.7
Anti-MHCII PE	eBiosciences	12-0920	clone HIS19
Anti-CD25 PE	eBiosciences	12-0251-82	clone PC61.5
Anti-CD62L PE	eBiosciences	12-0621-82	clone MEL-14
Anti-CD44 APC	eBiosciences	17-0441	clone IM7
Anti-IFN-gamma FITC	eBiosciences	11-7311-81	clone XMG1.2
Anti-IL-4 PE	BD Biosciences	554435	clone 11B11
Anti-IL-9 PE or APC	eBiosciences/Biolegend	50-8091-82/514104	clone RM9A4
Anti-IL-17a PE	BD Biosciences	559502	clone TC11-18H10
Anti-Foxp3 APC or PE	eBiosciences	17-5773-82/12-5773-80	clone FJK-16s
NaCl	Sigma	S7653	
KCl	Sigma	P9333	
Na ₂ HPO ₄ -2H ₂ O	Sigma	71643	
Dextrose/Glucose	Sigma	G7021	
HEPES, free acid	Sigma	H3375	
NH ₄ Cl	Sigma	A9434	
Disodium EDTA	Sigma	D2900000	
KHCO ₃	Sigma	237205	
CaCl ₂	Sigma	C5670	