

# Accessing Early Differentiation of Virus-Specific Follicular Helper CD4<sup>+</sup> T Cell in Acute LCMV-Infected Mice

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## Materials

Name	Company	Catalog Number	Comments
0.25% Trypsin-EDTA	Corning	25-052-CI	
4% Paraformaldehyde Fix Solution, 4% PFA	Beyotime	P0099-500mL	
70 µm cell strainer	Merck	CLS431751	
Alexa Fluor 647 anti-mouse TCR Va2 (clone B20.1)	Biologend	127812	1:200 dilution
Alexa Fluor 700 anti-mouse CD45.1 (clone A20)	Biologend	110724	1:200 dilution
APC anti-mouse CD25 (clone PC61)	Biologend	101910	1:200 dilution
B6 CD45.1 (B6.SJL-Ptprca Pepcb/BoyJ) mouse	The Jackson Laboratory	002014	
BeaverBeads Streptavidin	Beaver	22321-10	
Biotin anti-mouse F4/80 Antibody (clone BM8)	Biologend	123106	1:200 dilution
Biotin Rat anti-mouse CD11c (clone N418)	Biologend	117304	1:200 dilution
Biotin Rat anti-Mouse CD19 (clone 6D5)	Biologend	115504	1:200 dilution
Biotin Rat anti-Mouse CD8a (clone 53-6.7)	Biologend	100704	1:200 dilution
Biotin Rat anti-mouse NK-1.1 (clone PK136)	Biologend	108704	1:200 dilution
Biotin Rat anti-mouse TER-119/ Erythroid Cells (clone TER-119)	Biologend	116204	1:200 dilution
bovine serum albumin, BSA	Sigma	A7906	

Brilliant Violet 421 anti-T-bet (clone 4B10)	Biolegend	644816	1:100 dilution
Brilliant Violet 605 anti-mouse CD279 (PD-1) (clone 29F.1A12)	Biolegend	135220	1:200 dilution
C57BL/6J (B6) mouse	The Jackson Laboratory	000664	
CXCR5-GFP knock-in reporter mouse			In house; the CXCR5-GFP knock-in mouse line was generated by the insertion of an IRES-GFP construct after the open reading frame of Cxcr5.
DMEM 10% medium			DMEM medium containing 10% FBS
DMEM medium	Gibco	11885092	
EDTA	Sigma	E9884	
FACSFortesa	BD Biosciences		
Fetal bovine serum, FBS	Sigma	F8318	
FlowJo (version 10.4.0)	BD Biosciences		
Foxp3/Transcription Factor Staining Buffer Set	Invitrogen	00-5523-00	The kit contains three reagents: a. Fixation/Permeabilization Concentrate (4X); b. Fixation / Permeabilization Diluent; c. Permeabilization Buffer.
Goat Anti-Rat IgG Antibody (H+L), Biotinylated	Vector laboratories	BA-9400-1.5	1:200 dilution
Invitrogen EVOS FL Auto Cell Imaging System	ThermoFisher Scientific		
Isolation buffer			FACS buffer containing 0.5% BSA and 2mM EDTA
LCMV GP61-77 peptide (GLKGPDIYKGVYQFKSV)	Chinese Peptide Company		
LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit, for 633 or 635 nm excitation	Life Technologies	L10199	1:200 dilution
MigR1	addgene	#27490	
NaN3	Sigma	S2002	
Opti-MEM medium	Gibco	31985070	
pCL-Eco	addgene	#12371	
PE anti-mouse CD69 (clone H1.2F3)	Biolegend	104508	1:200 dilution
PE Mouse anti-Bcl-6 (clone K112-91)	BD Biosciences	561522	1:50 dilution
Phosphate buffered saline, PBS	Gibco	10010072	
Polybrene	Solarbio	H8761	
Purified Rat Anti-Mouse CXCR5 (clone 2G8)	BD Biosciences	551961	1:50 dilution
Rat monoclonal PerCP anti-mouse CD4 (clone RM4-5)	Biolegend	100538	1:200 dilution
recombinant murine IL-2	Gibco	212-12-1MG	
Red Blood Cell Lysis Buffer	Beyotime	C3702-500mL	
RPMI 1640 medium	Sigma	R8758	
RPMI 2%			RPMI 1640 medium containing 2% FBS
SMARTA (SM) TCR transgenic mouse			SM TCR transgenic line in our lab is a gift from Dr. Rafi Ahmed (Emory University). Additionally, this mouse line can also be obtained from The Jackson Laboratory (stain#: 030450).
Staining buffer			PBS containing 2% FBS and 0.01% NaN3

Streptavidin PE-Cyanine7	eBioscience	25-4317-82	1:200 dilution
TCF1/TCF7 (C63D9) Rabbit mAb (Alexa Fluor 488 Conjugate)	Cell signaling technology	6444S	1:400 dilution
TFH cell staining buffer			FACS buffer containing 1% BSA and 2% mouse serum
TransIT-293 reagent	Mirus Bio	MIRUMIR2700	