

T helper cell subset	Day 0 conditions	Day 1 conditions	Day 0 - Day 3 concentrations (on stimulation)	Day 3 (expansion)	Day 3 - Day 5 concentrations (not on stimulation)
	1mL/well final volume (0.5mL/well mastermix + 0.5mL/well cells in cMDM following isolation); cells plated at 300,000-500,000 cells/well	2mL/well final volume (1mL/well mastermix is added on D1)		2mL/well final volume (1mL/well mastermix + 1mL/well cells in cMDM)	
Media:	0.5mL/well cMDM	1mL/well cMDM		1mL/well cMDM	
Th0	10uL/well anti-Ifng (1ug/uL stock) 10uL/well anti-IL-4 (1ug/uL stock)	10uL/well anti-Ifng (1ug/uL stock) 10uL/well anti-IL-4 (1ug/uL stock)	10ug/mL anti-Ifng 10ug/mL anti-IL-4	8uL/well IL-2 (Peprotech 1ng/uL stock)	1:2 4ng/mL IL-2
Th1	5uL/well anti-IL-4 (1ug/uL stock)	1uL/well IL-12 (10ng/uL stock) 5uL/well anti-IL-4 (1ug/uL stock) 40uL/well IL-2 (Peprotech, 1ng/uL stock)	5ng/mL IL-12 5ug/mL anti-IL-4 20ng/mL IL-2	1uL/well IL-12 (10ng/uL stock) 5uL/well anti-IL-4 (1ug/uL stock) 40uL/well IL-2 (Peprotech, 1ng/uL stock)	1:2.5 5ng/mL IL-12 2.5ug/mL IL-4 20ng/mL IL-2
Tfh-like	Th1	Th1	same as Th1	1uL/well IL-12 (10ng/uL stock) 5uL/well anti-IL-4 (1ug/uL stock) 1.6uL/well IL-2 (Peprotech, 1ng/uL stock)	1:2.5 5ng/mL IL-12 2.5ug/mL anti-IL-4 0.8ng/mL IL-2
Th2	10uL/well anti-Ifng (1ug/uL stock) 40uL/well IL-2 (Peprotech, 1ng/uL) 2uL/well IL-4 (10ng/uL stock)	10uL/well anti-Ifng (1ug/uL stock) 40uL/well IL-2 (Peprotech, 1ng/uL) 2uL/well IL-4 (10ng/uL stock)	10ug/mL anti-Ifng 20ng/mL IL-2 10ng/mL IL-4	20uL/well anti-Ifng (1ug/uL stock) 40uL/well IL-2 (Peprotech, 1ng/uL) 2uL/well IL-4 (10ng/uL stock)	1:2.5 10ug/mL anti-Ifng 20ng/mL IL-2 10ng/mL IL-4
Th17	10uL/well anti-Ifng (1ug/uL stock) 10uL/well anti-IL-4 (1ug/uL stock) 1uL/well IL-6 (50ng/uL stock) 1uL/well TGFb (3ng/uL stock)	10uL/well anti-Ifng (1ug/uL stock) 10uL/well anti-IL-4 (1ug/uL stock) 1uL/well IL-6 (50ng/uL stock) 1uL/well TGFb (3ng/uL stock)	10ug/mL anti-Ifng 10ug/mL anti-IL-4 50ng/mL IL-6 3ng/mL TGFb	2uL/well IL-6 (50ng/uL stock) 3.2uL/well (Peprotech 1ng/uL stock)	1:2 50ng/mL IL-6 1.6ng/mL IL-2
Treg	10uL/well anti-Ifng (1ug/uL stock) 10uL/well anti-IL-4 (1ug/uL stock) 0.67uL/well TGFb (3ng/uL stock)	10uL/well anti-Ifng (1ug/uL stock) 10uL/well anti-IL-4 (1ug/uL stock) 0.67uL/well TGFb (3ng/uL stock)	10ug/mL anti-Ifng 10ug/mL anti-IL-4 2ng/mL TGFb	8uL/well IL-2 (Peprotech 1ng/uL stock)	1:2 4ng/mL IL-2

Cytokine/antibody details	Source	Catalog#	Prepared or purchased Concentration
anti-IL-4, clone 11B11	BioLegend	504115	1ug/uL
anti-Ifng, clone XMG1.2	BioLegend	505827	1ug/uL
anti-TGFb, clone 1D11	BioXcell	BE0057-A005	6.9ug/uL
anti-IL-2, clone JES6-1A12	eBioscience/Fisher	16-7022-85	1ug/uL
IL-12	R&D	419-ML	10ng/uL
IL-2	Peprotech	200-02	stock @ 100ng/uL, working @ 1ng/uL (in cMDM)
IL-4	R&D	404-ML	10ng/uL
TGFb	Gibco	PHG9204	3ng/uL
IL-6	R&D	406-ML	50ng/uL
IL-21	Peprotech	210-21	50ng/uL

Note that for Peprotech IL-2: 10uL of 100ng/uL stock is diluted in 1mL cMDM and incubated on ice for 15 minutes before use. This is good for 1 week.