Current approaches to MHC class II tetramer staining

It should be appreciated that the development and utilization of class II tetramers is still in a largely experimental stage. A brief review of the literature along with our own observations suggests that there may not be any definitive protocol for staining with all MHC class II tetramers. (This is particularly true for mouse tetramers.) Among published reports and feedback from our clients, class II tetramer staining reactions have been carried out at a range of temperatures (4°C to 37°C), concentrations (1-20 ug/ml) and times (20 min to 20 hours). A number of investigators have recently published good results using 3 hours at 37°C. Additionally, there are reports that cross-linking TCRs on the target cell surface prior to tetramer staining has significant benefits in some cases (for good examples of the above mentioned staining parameters, see Falta et. al., 2005, Arthritis and Rheumatism, vol 52 No. 6 pp1885-1896).

We have come up with a suggested staining optimization (Table 1). We strongly urge all investigators to perform this optimization for any untested reagent. Please note that it is *critically important* to have a negative control tetramer with an irrelevant peptide to rule out non-specific staining. We typically provide an hCLIP-containing tetramer for these purposes. In the very rare case that we do not, we suggest that you use biological negative controls such as a population of cells that you know should not have cells of the specificity of the reagent ordered. Additionally, culture of lymphocytes at 37°C for several hours results in a significant number of dead cells, which should be excluded during analysis as they often non-specifically bind up tetramer. We use propidium iodide for live/dead discrimination at a final concentration of 0.5 ug/ml and find this works extremely well. Good flow cytometry technique is essential for users of class II tetramers.

We stand ready to help wherever possible. When contacting us for assistance, please remit all optimization data by email first. For technical assistance with these reagents contact:

Rick Willis, Ph. D., Technical Director
404-727-7215 or richard.willis@emory.edu

Table 1. Conditions for optimization of MHC class II tetramer staining recommended by TCF for all new reagents.

	4 deg C		37 deg C	
	Experimental	**Control**	**Experimental**	**Control**
0.5 hrs	2ug/ml	2ug/ml	2ug/ml	2ug/ml
	6ug/ml	6ug/ml	6ug/ml	6ug/ml
	18ug/ml	18ug/ml	18ug/ml	18ug/ml
1.5 hrs	2ug/ml	2ug/ml	2ug/ml	2ug/ml
	6ug/ml	6ug/ml	6ug/ml	6ug/ml
	18ug/ml	18ug/ml	18ug/ml	18ug/ml
3.0 hrs	2ug/ml	2ug/ml	2ug/ml	2ug/ml
	6ug/ml	6ug/ml	6ug/ml	6ug/ml
	18ug/ml	18ug/ml	18ug/ml	18ug/ml

Table 2. Optimal Staining Conditions Reported by Clients

Tetramer	Time	Temp	Dilution	Other
I-A(b) Ova 323	2h	37ºC	1:10	RPMI with 10% serum; 100 uL staining volume (or less); Does not stain 100% of appropriate cells
I-A(b) MOG 38	20h *or* 3h[a] *or* 3h[b] *or* 0.5h[c]	37ºC *or* 37ºC[a] *or* 4ºC[b] *or* 4ºC[c]	? *or* 1:100[a] *or* 1:50[b] *or* 1:300[c]	Stains polyclonal cells primed with peptide and adjuvant; Does not stain all antigen-reactive cells; Does not stain 2D2 Tg; complete RPMI with 10% serum[a] or PBS[b] or DMEM with 10% FCS[c]
I-A(b) FMLV	3h	37ºC	1:400	Supplemented RPMI media; 1.2×10^6 cells
I-A(b) LTAg	2h	37ºC	1:100	RPMI with 2% FCS
I-A(b) MHV	1h	37ºC	1:220	RP10 media
I-A(b) ESAT-6	1h	37ºC	1:50	RPMI with 10% FCS and monensin
I-A(b) LLO	2h	37ºC	1:50	Staining done without FC block

A Case Study: IA^b gp66-77 Tetramers

For the IA^b gp66-77 tetramers, staining conditions have been worked out in some detail. Optimal staining was seen with 2 ug/ml tetramer at 37ºC (figure 1). At 4ºC, very little staining was seen at any tetramer concentration. In a separate experiment, it was found that significant staining took place within 30 mins, but longer incubations led to slight increases (figure 2). Based on these data, we recommend 3 hours @ 37ºC with a tetramer concentration of 2 ug/ml for the IA^b-gp66-77 tetramer.

Figure 1.

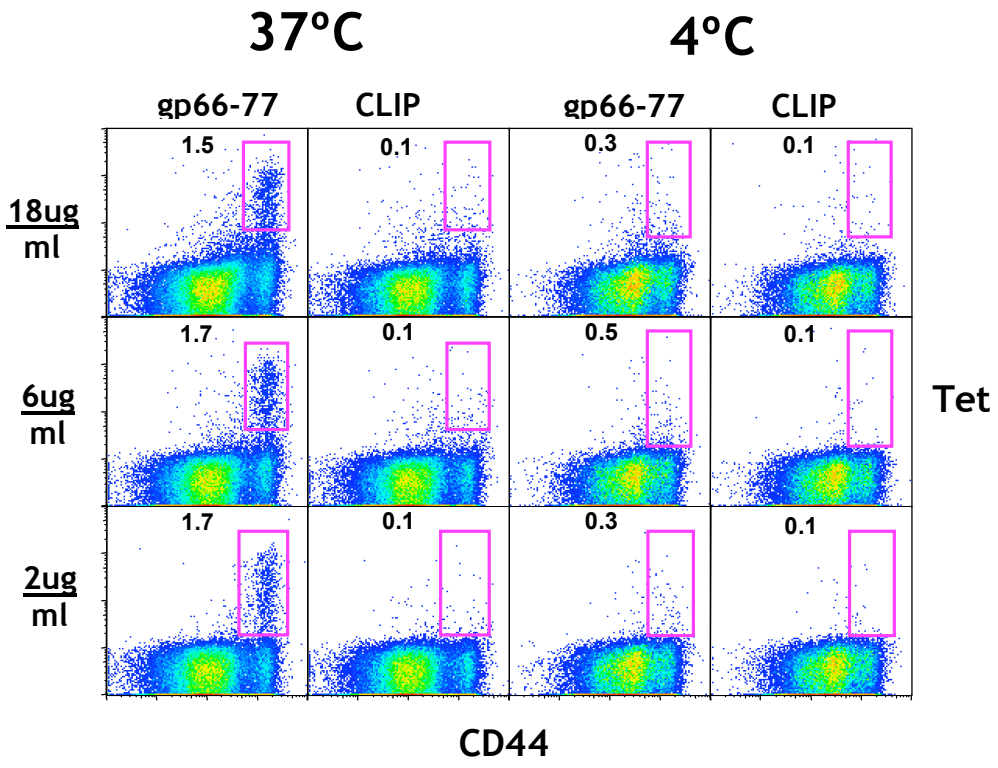

Figure 1. Optimization of IA^b-gp66-77 tetramer staining with varying temperature and tetramer concentration. Splenocytes at 8 days post infection with LCMV Armstrong were stained for 3 hrs at either 37ºC or 4ºC in 1.5 ml tubes with varying concentrations of tetramer. Cells were then washed and stained with mAbs for CD4, CD8, and CD44 and with propidium iodide (PI). Events were gated on lymphocytes by light scatter characteristics, then for CD4+ CD8- PI- events. Gates were drawn by setting the control tetramer gate to 0.1% and using this gate for the corresponding gp66-77 sample. Numbers are the percentage of tetramer-positive events among all CD4 cells.

Figure 2.

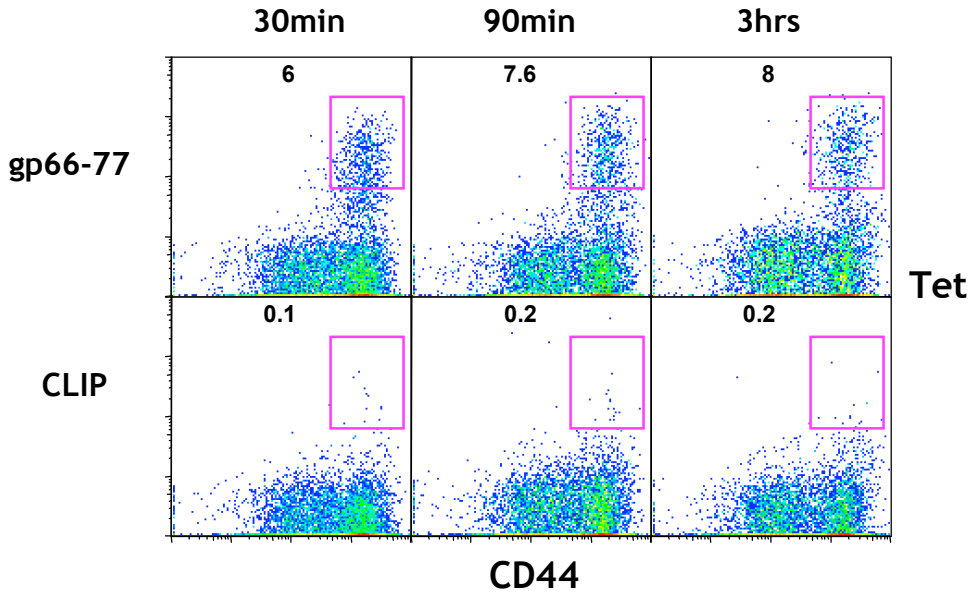

Figure 2. Optimization of IA^b-gp66-77 tetramer staining with varying length of incubation. Splenocytes at 8 days post infection with LCMV Armstrong were stained at 37ºC for varying amounts of time with either gp66-77 or Clip tetramers. Tetramer concentration was 2 ug/ml. Cells were then washed and stained with mAbs for CD4, CD8, and CD44 and with propidium iodide (PI). Events were gated on lymphocytes by light scatter characteristics, then for CD4+ CD8- PI- events. Numbers are the percentage of tetramer-positive events among all CD4 cells.

Figure 3.

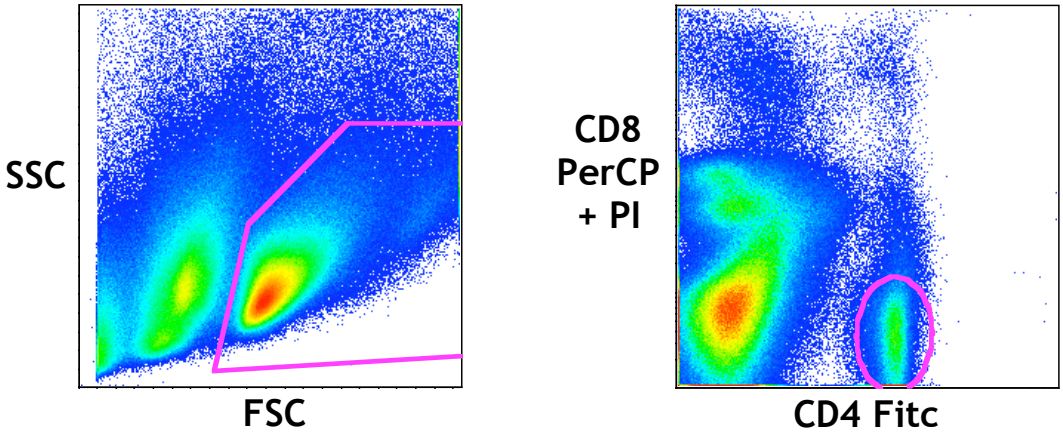

Figure 3. Gating strategy. For all data, cells were stained with CD4-FITC, Tet-PE, CD8a-PerCP, PI and CD44-APC. Data was acquired on a BD FACSCalibur. (Note that both PerCP and PI emit in the FL3 channel on this instrument.) Events were gated on lymphocytes by light scatter characteristics, then for CD4+ CD8- PI- events.