FHCRC HVTN Laboratory
ICS Assay Study Specific Procedures for PTE-A Peptide Validation December 2009

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1. Purpose

1.1 These study specific procedures are to be used in conjunction with the ICS Assay, SOP # FH-HVTN-A0002.GEN and are specific to the study or protocol indicated. Details of the specimens, peptides, and other protocol-specific procedures are described herein.

2. Procedure – Day 2

2.1 Prepare cells

2.1.1 For the plate map specified in Attachment 3, 19 million cells are required to plate 1 million cells per well.

2.1.2 If fewer cells are recovered, the cells may be plated at fewer than 1 million cells per well, to a minimum of 500,000 cells/well. Thus a minimum of 9.5 million cells is required to test all of the peptide pools. If fewer than 19 million but at least 9.5 million cells are recovered, resuspend the cells in 1.2 ml media and record this on the cell thawing worksheet.

2.1.3 These cells will also be used in the ELISpot assay, which will require 8.4 million cells. So a minimum of 17.9 million cells total are required to do both assays. If fewer than 17.9 million cells are recovered, perform the ELISpot assay if there are at least 8.4 million cells, and do not plate the sample for ICS.

2.2 Preparing stimulation cocktails: refer to the ICS Stimulation Cocktail Worksheet (Attachment 1).

2.4 Preparing antibody cocktail: refer to the ICS Antibody Cocktail Worksheet (Attachment 2).

Attachment 1

ICS Stimulation Cocktail Worksheet

Stimulation Cocktails:

Stim condition	Stock conc. (Dilution in mix)	Volume peptide or DMSO (uL)	Volume CD28/49d use at 1:10 (uL)	Volume of BfA use at 1:5 (uL)	Volume of PBS (uL)	Final volume (uL)	Performed by
Neg Ctrl (DMSO)	Stock (1:20)	26	52	104	338	520	
CMV	40 ug/ml (1:4)	60	24	48	108	240	
SEB	0.5mg/ml (1:50)	6	30	60	204	300	
For the HIV peptide pools, prepare a master mix containing CD28/49d, BfA and PBS using the volumes below.							
Master Mix	N/A	N/A	315	630	2205	3150	
PTE-A Env 1	100ug/ml (1:10)	40	Use 360ul of the Master Mix			400	
PTE-A Env 2	100ug/ml (1:10)	40	Use 360ul of the Master Mix			400	
PTE-A Env 3	100ug/ml (1:10)	40	Use 360ul of the Master Mix			400	
PTE-A Gag 1	100ug/ml (1:10)	20	Use 180ul of the Master Mix			200	
PTE-A Gag 2	100ug/ml (1:10)	20	Use 180ul of the Master Mix			200	
PTE-A Nef 1	100ug/ml (1:10)	20	Use 180ul of the Master Mix			200	
PTE-A Pol 1	100ug/ml (1:10)	20	Use 180ul of the Master Mix			200	
PTE-A Pol 2	100ug/ml (1:10)	20	Use 180ul of the Master Mix			200	
PTE-A Pol 3	100ug/ml (1:10)	20	Use 180ul of the Master Mix			200	
PTE-A Rev/Vpr 1	100ug/ml (1:10)	20	Use 180ul of the Master Mix			200	
PTE-A Tat/Vpu 1	100ug/ml (1:10)	20	Use 180ul of the Master Mix			200	
PTE-A Vif 1	100ug/ml (1:10)	20	Use 180ul of the Master Mix			200	

Attachment 2

ICS Antibody Cocktail Worksheet

Preparation of antibody cocktail

Use the AViD live/dead stain with this panel

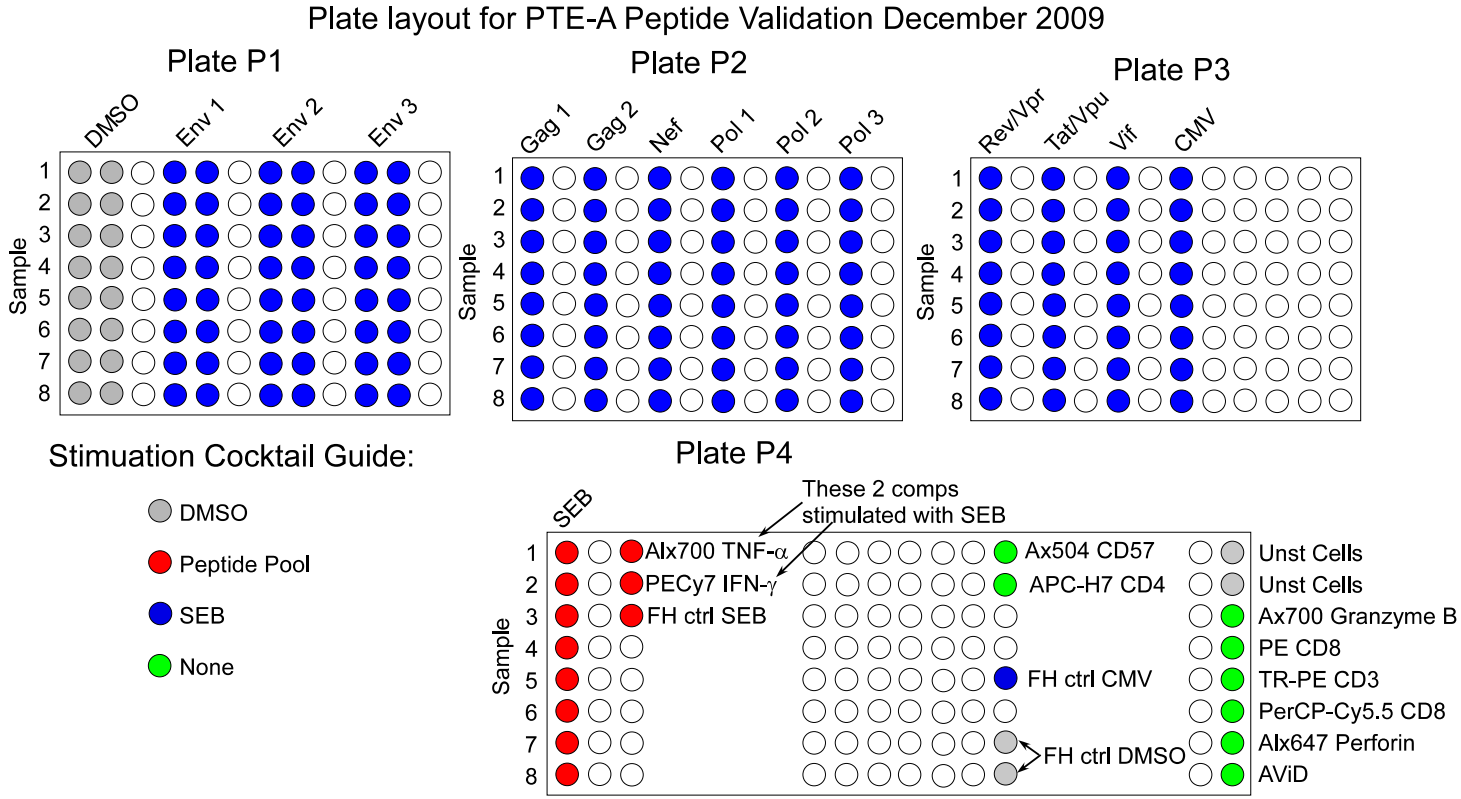
Number of tests required calculated as:

Number of PBMC samples_____**x 19 + 4** (for FH Ctrl wells) **=**_____

				For comp wells		For cocktail	
Cocktail	**Lot#**	**Exp date**	**Titre (µl)**	**Comp well (µl)**	**By**	**Volume (µl) to add to mix**	**By**
FITC TNF							
PE IL2				**Use PE CD8 below**			
TR-PE CD3							
PerCP-Cy5.5 CD8							
PE-Cy7 IFNγ							
Ax647 Perforin*							
Ax700 Granzyme B							
Ax405 CD57							
APC-H7 CD4							
PE CD8						N/A	N/A
					Total Ab Volume (ul):		N/A
					FACS Wash Volume (ul):		
					Total stain cocktail volume (ul): = #tests x 55		N/A

Attachment 3

Plate Layout



After staining is complete, add 150ul PBS, FACS Wash, or 1% PfA to the columns to the right of DMSO and the peptide pool columns in plates 1, 2, and 3; to the columns to the right of the SEB wells in plate 4; to the wells beneath FH ctrl CMV, FH ctrl SEB, and APC-H7 CD4 in plate 4.