Custom Peptide Mixes for Cytokine Flow Cytometry

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Introduction

Mixtures of overlapping peptides have been increasingly used for *in vitro* stimulation of T cells.[1-3] Stimulated T cells can be measured and enumerated by cytokine flow cytometry (CFC), ELISPOT, or other monitoring assays. CFC is a particularly beneficial technology as it allows one to differentiate responses of CD4 and CD8 T cells, which are readily resolved by the multiparametric nature of flow cytometry.

Overlapping peptide mixtures have several benefits over the use of whole protein and whole viral lysate preparations. First, because they are synthetically derived, they can be made reproducibly, with less potential lot-to-lot variability. Second, whole protein antigens preferentially stimulate MHC class II–restricted $CD4^+$ T-cell responses.[4,5] This is because of the tendency of exogenous antigens to be processed for presentation by MHC class II–molecules. By carefully choosing the peptide length, overlapping peptide mixtures can stimulate both CD4 and CD8 responses in the same sample.

Mixtures of 15-mer peptides that overlap by 11 amino acid residues each can span an entire immunogenic protein so that all possible epitopes of 9 amino acid residues are contained in at least one peptide of the mixture. The mixture can then be used as a single antigen for short in vitro restimulation (about 6 hours) of peripheral blood mononuclear cells (PBMCs) or whole blood. Alternately, smaller pools of overlapping peptides can be created using a "matrix" approach, so that epitopes can be rapidly mapped.[1,6]

The synthetic peptide mixtures bind to extracellular MHC molecules and directly stimulate T cells. CD8 T cells respond to peptides that are 8–10 amino acid residues in length. The binding groove of MHC class I–molecules has "closed" ends that make it difficult for longer peptides to bind.[7,8] CD4 cells can respond to peptides of 10 amino acid residues or longer, as the binding groove of MHC class II–molecules has open ends that allow longer peptides to protrude.[9] Despite these differences in length requirements for MHC class I– and MHC class II–binding, peptides of 15 amino acid residues have been shown to efficiently stimulate both CD4 and CD8 responses.[3] The mechanism by which 15-mer peptides can elicit CD8 responses is not fully understood, although the responses can be shown to be MHC class I–restricted. It is assumed that extracellular processing by serum or cell-associated proteases, or both, plays a role. However, this mechanism appears to be limited, as 20-mer peptides are less efficient than 15-mers at inducing CD8 responses[3] (see Figure 1).

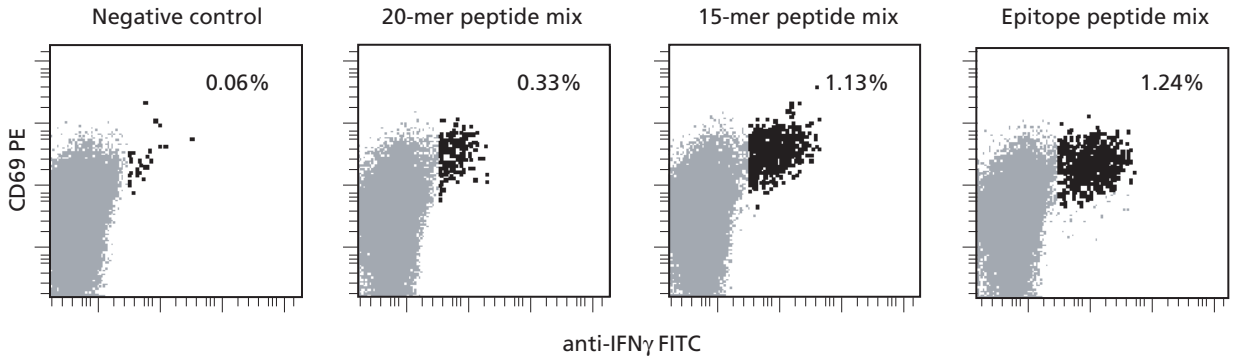

Figure 1. *Comparison of CD8 responses using an overlapping peptide mix of 20-mer peptides, 15-mer peptides, or known 8–12 amino acid epitopes from HIV p55 gag, in an HIV seropositive donor.*

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Materials and Methodology

BD FastImmune™ CD8 (IFN-γ, four-color) and CD4 (IFN-γ, IL-2, TNF-α, three-color) Cytokine Detection Kits readily identify CD4 and CD8 responses in human whole blood or PBMC samples stimulated with peptide mixes.* The kits make getting started easier with a simplified protocol and optimized staining and sample processing reagents (see Figure 2). The three-color BD FastImmune CD4 Kit Multicolor Cocktail (Anti-Cytokine FITC/CD69 PE†/CD4 PerCP-Cy5.5†) can be supplemented with CD3 APC† to allow further discrimination of $CD3^+CD4^+$ cells from $CD3^+CD4^-$ cells. The latter population is roughly equivalent to direct detection of CD3+CD8+ cells. However, $CD4^+CD8^{dim}$ T cells (which are MHC class II–restricted[10]) will be included in the $CD3^+CD8^+$ population, but not the $CD3^+CD4^-$ population. Please note that the BD FastImmune system is highly optimized to allow for rare event detection and should not be mixed with other reagent products.

Kits contain:

- BD FastImmune Anti-Hu–IFN-γ FITC/CD69 PE*/CD8 PerCP-Cy5.5*/CD3 APC*
  Or BD FastImmune Anti-Hu–cytokine FITC/CD69 PE/CD4 PerCP-Cy5.5
- BD FastImmune matching multicolor isotype control
- BD FastImmune Brefeldin A
- BD FastImmune EDTA Solution
- BD FastImmune CD28/CD49d Costimulatory Reagent
- BD FACS™ Lysing Solution†
- BD FACS™ Permeabilizing Solution 2

Note: All kit components are also available individually.

In addition to the ability to simultaneously detect CD4 and CD8 responses in a single sample, peptide mixes also allow for better detection of responses in cryopreserved PBMCs or shipped whole blood.[3] This seems to reflect the fact that antigen-presenting cells, such as monocytes, are more affected by shipping, or freezing or thawing, or both, than are T cells. Thus, use of peptide mixes can allow flexibility in clinical research studies for which samples must be shipped or cryopreserved prior to analysis.

The complexity of peptide mixtures can be extensive, especially for large proteins, requiring 100 or more peptides to span the entire protein. To help facilitate the use of peptide mixes for CFC and other assays, BD Biosciences now offers peptide mixes for selected antigens through their custom program. Two such mixes, for CMV pp65 and HIV p55 gag, are available in stock and can be ordered in small volumes. Examples of responses to these peptide mixes are shown in Figure 3.

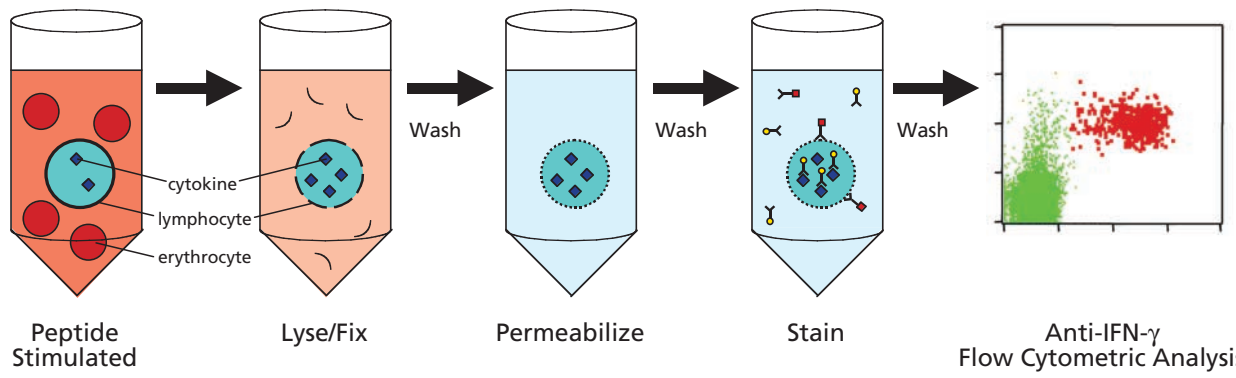

Figure 2. Overview of BD FastImmune assay for antigen-specific results in hours.

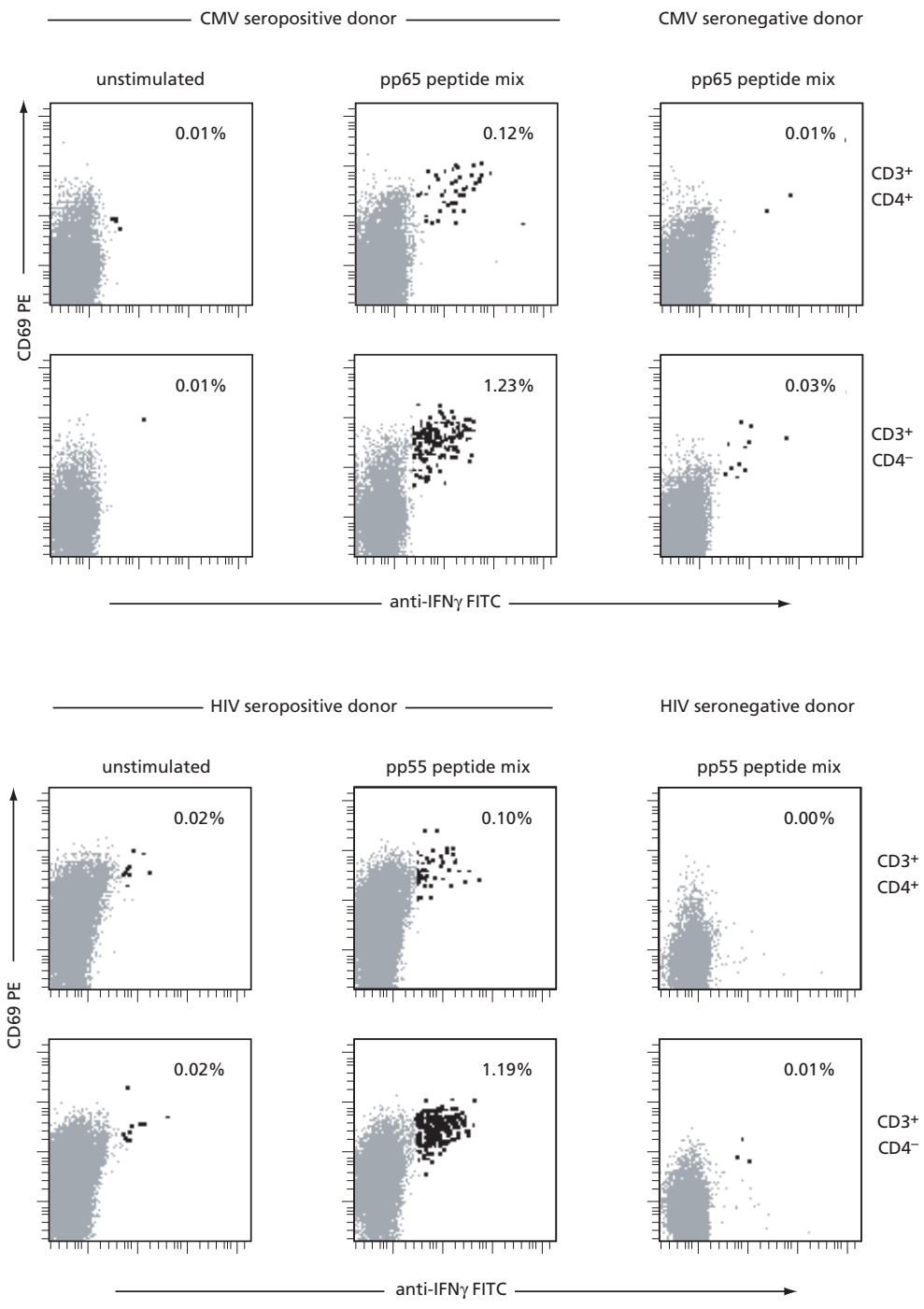

Figure 3. (A) Representative responses to CMV pp65 peptide mix in a CMV seropositive versus CMV seronegative donor. (B) Representative responses to HIV p55 gag peptide mix in an HIV seropositive versus HIV seronegative donor.

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In conclusion, the use of peptide mixes of 15-mers overlapping by 11 amino acid residues appears to be efficient for detection of total CD4 and CD8 responses to an entire protein. Because the peptides are synthetically derived, they might offer more lot-to-lot stability than recombinant proteins or whole virus preparations. In addition, they appear optimally compatible with cryopreserved PBMCs or shipped whole blood. Thus, clinical research studies evaluating immunogenicity of vaccine preparations might benefit from use of such peptide mixes and CFC analysis.

Supporting Literature and Workshops

Please contact your local BD Biosciences representative for more detailed information.

- BD FastImmune CFC Handbook
  - Performance Characteristics of Antigen-Specific Cytokine Flow Cytometry
- BD FastImmune Application Notes
  - Cytokine Detection in Antigen-Activated $CD8^+$ and $CD4^+$ T cells
  - Simultaneous Detection of Proliferation and Cytokine Expression in Peripheral Blood Mononuclear Cells
- Intracellular Cytokine Brochure (complimentery featuring other than BD FastImmune intracellular reagents and protocols)
- Intracellular Cytokine Workshop

References:

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2. Kern F, Faulhaber N, Frommel C, et al. Analysis of CD8 T cell reactivity to cytomegalovirus using protein- spanning pools of overlapping pentadecapeptides. Eur J Immunol. 2000;30:1676.
3. Maecker HT, Dunn HS, Suni MA, et al. Use of overlapping peptide mixtures as antigens for cytokine flow cytometry. J Immunol Methods. 2001;255:27.
4. Braciale TJ, Morrison LA, Sweetser MT, Sambrook J, Gething MJ, and Braciale VL. Antigen presentation pathways to class I and class II MHC-restricted T lymphocytes. Immunol Rev. 1987;98:95.
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6. Kern F, Surel IP, Brock C, et al. T-cell epitope mapping by flow cytometry. Nat Med. 1998;4:975.
7. Bjorkman, PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, and Wiley DC. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. Nature. 1987;329:512.
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9. Bjorkman, PJ, and Burmeister WP. Structures of two classes of MHC molecules elucidated: crucial differences and similarities. Curr Opin Struct Biol. 1994;4:852.
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Custom Peptide Mixes & Cytokine Kits Product List

AVAILABLE CUSTOM PEPTIDE MIXES	SIZE	CAT. NO.	PRICE
CMV pp65 peptide mix (138 Peptides)	5 Test[a]	**551969**	$125
HIV p55 gag peptide mix (127 Peptides)	5 Test[a]	**551940**	$125
CEA peptide mix (173 Peptides)	5 Test[a]	**552821**	$125

[a] Each test is sufficient for activation of 1 mL whole blood or 1 mL PBMC at 1 × 10^6 cells/mL.

BD FASTIMMUNE CYTOKINE KITS	FORMAT	SIZE	CAT. NO.	PRICE
CD8 Anti-Hu–IFN-γ Intracellular Detection Kit	FITC/PE/PerCP-Cy5.5/APC	25 tests each for	**346049***	$995
CD4 Anti-Hu–IFN-γ Intracellular Detection Kit		stimulated and	**340970***	
CD4 Anti-Hu–IL-2 Intracellular Detection Kit	FITC/PE/PerCP-Cy5.5	resting samples	**340971***	$895
CD4 Anti-Hu–TNF-α Intracellular Detection Kit			**340972***	
BD FastImmune Anti-Hu–IFN-γ /CD69/CD8/CD3	FITC/PE/PerCP-Cy5.5/APC	50 tests	**346047***	$650
BD FastImmune Isotype Control IgG2a/IgG1/CD8/CD3	FITC/PE/PerCP-Cy5.5/APC	50 tests	**346048**	$445
BD FastImmune Anti-Hu–IFN-γ/CD69/CD4	FITC/PE/PerCP-Cy5.5	50 tests	**340962***	$620
BD FastImmune Anti-Hu–IL-2/CD69/CD4	FITC/PE/PerCP-Cy5.5	50 tests	**340963***	$620
BD FastImmune Anti-Hu–TNF-γ/CD69/CD4	FITC/PE/PerCP-Cy5.5	50 tests	**340964***	$620
BD FastImmune Isotype Control IgG2a/IgG1/CD4	FITC/PE/PerCP-Cy5.5	50 tests	**340965**	$415
Use during Sample Activation				
BD FastImmune CD28/CD49d Costimulatory Reagent, 1×[a]		300 mL	**347690**	$135
BD FastImmune Brefeldin A Solution, 10×[b]		250 mL	**347688**	$135
BD FastImmune EDTA Solution, 1×[c]		2.50 mL	**347689**	$75
Use for Lysis and Permeabilization Post Sample Activation				
BD FACS Lysing Solution, 10× (150 tests standard protocol)		30 mL	**347691**	$105
BD FACS Lysing Solution, 10× (500 tests standard protocol)		100 mL	**349202**	$345
BD FACS Permeabilizing Solution 2, 10× (200 tests)		10 mL	**347692**	$170
BD FACS Permeabilizing Solution 2, 10× (500 tests)		25 mL	**340973**	$430

[a] Use at 5 mL/0.5 mL whole blood.
[b] Dilute 1:10 with sterile PBS and use at 1× concentration, 10 mL/0.5 mL whole blood.
[c] Use at 1× concentration at 50 mL/0.5 mL whole blood.

All products in this table are manufactured under cGMP

* Use of these products to measure activation antigens expressed on mononuclear cell subsets for purpose of monitoring immunoregulatory status can fall under one or more claims of the following patents: US 5,445,939; 5,656,446; 5,843,689; Europe 319,543; Canada 1,296,622; Australia 615,880; and Japan 2,769,156.

† Patents—PE and APC: US 4,520,110; 4,859,582; 5,055,556; Europe 76,695; Canada 1,179,942 PerCP: US 4,876,190 Cy5.5: US 5,268,486; 5,486,616; 5,569,587; 5,569,766; 5,627,027
BD FACS Lysing Solution: US 4,654,312; 4,902,613; 5,098,849