SpotMix™ Peptide Pools: a New Approach for Rapid Antigen Target Identification

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Introduction

Enhancing specific immune responsiveness by vaccination is a promising therapeutic approach to infectious diseases and cancer. Its success, however, depends on the identification of suitable target antigens and epitopes. Stimulation with intact proteins or whole protein-spanning, overlapping peptide pools (PepMix™) has been successfully used for that purpose. However, this approach comes with a significant effort in terms of time, labor and cost, in particular if many potential candidate proteins have to be analyzed.

Based on a method for the highly parallel synthesis of multiple peptides in low quantities, we developed a novel protocol which permits the production of equimolar pools of SPOT peptides the so called SpotMix™ peptide pools.

Here, we show examples comparing classic PepMix™ peptide pools and SpotMix™ peptide pools in T-cell stimulation assays like ELISpot and flow cytometry/intracellular cytokine staining (ICS).

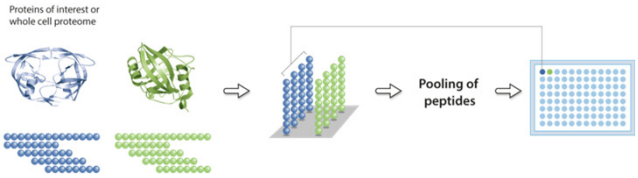

Figure 1: **Peptide Pool concept**. Overlapping 15-mer peptides covering the whole length of the protein of interest can be used for different functional T-cell assays like ELISpot and ICS.

Methods

PepMix™ peptide pools are manufactured using classical solid phase peptide synthesis on polystyrene resins. Peptides purification is performed by RP-HPLC. Purity is determined by HPLC-MS analysis.

SpotMix™ peptide pools **:** Synthesis is performed via fully automated SPOT-synthesis approach. Peptides are cleaved into Microtiter-plate wells, analyzed and quantified by LCMS and spin dried. Subsequently, the peptides are pooled and aliquoted.

SpotMix™ PLUS are synthesized the same way with 100% quality controls (see also Table 1).

For this study the following proteins were produced as PepMix™ peptide pools and SpotMix™ PLUS peptide pools : UL83, UL55, EBNA1, EBNA3, BZLF1.

Ex-vivo ELISpot: Cryopreserved PBMCs were thawed and rested overnight Subsequently 2-3 x10^5 cells were either left untreated or stimulated with peptides for 16-20h. Each stimulant was tested in triplicates. The secretion of IFNγ was detected using ALP coupled antibody and BCIP/NBT as substrate. Developed ELISpot plates were scanned using AID iSpot reader and analyzed with corresponding software.

FACS/ICS: Cryopreserved PBMCs were thawed and rested overnight . 2x10^5 cells were either left untreated or stimulated with peptides for 16h in the presence of Brefeldin A to inhibit cytokine secretion. After permeabilization and fixation, the cells were stained with fluorochrome-coupled antibodies against CD3, CD4, CD8 and IFNγ. Flow cytometry was performed on a BD accuri C6 and data analyzed with FlowJo X software.

Table 1 Comparative overview defining the different peptide pool approaches.

	SpotMix™	SpotMix™ PLUS	PepMix™
Amount/Peptide	Varying (approx. 5-20 µg)	10 µg /peptide (each peptide quantified)	From 1mg/ peptide (each peptide quantified)
Peptide Purity	No purification	Purified by dialysis, if >20 peptides/pool	Unpurified or purified by HPLC up to GxP grade
Peptide QC	5% peptides analyzed by LC-MS	100% peptides analyzed by LC-MS	100% peptide analyszed by LC-MS
Pool QC	None	none	Full QC for each peptide pool
Applications	**Antigen discovery**	**Neo-epitope based immune monitoring, Antigen discovery**	**Clinical immune monitoring, preclinical & clinical research, immunotherapy**

Results

Two Cytomegalovirus (CMV) positive donors (#D1 and #D2) were analyzed for Interferon γ (IFNγ) secretion upon stimulation with either synthetic UL83 PepMix™ or UL83 SpotMix™ PLUS in the ex-vivo ELISpot setting (Figure 2 and 3) and using intracellular cytokine staining (Figure 4).

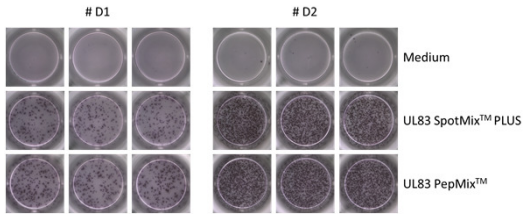

Figure 2: **Ex-vivo ELISpot data.** Exemplary wells of stimulation of donor #1 and #2 are shown.

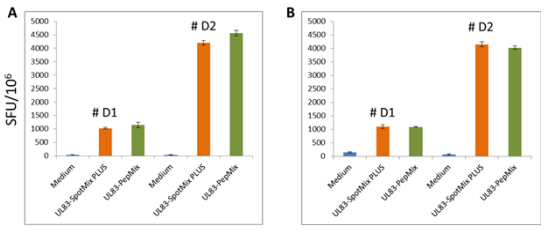

Figure 3: **Comparison of two independent SpotMix™ PLUS synthesis.** A and B present quantified data obtained using two different Lots of UL83 SpotMix™ PLUS in ELISpot experiments. Shown are mean values of triplicates (and standard deviation) presented as spot forming units (SFU) per Million cells.

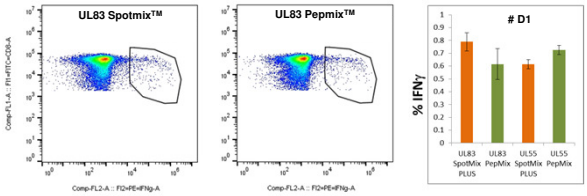

Figure 4: **Comparative ICS.** Left side: Exemplary ICS data with quantification of IFNγ positive cells (of cytotoxic T-lymphocytes) is depicted. The bar plots on the right side represent the mean values of duplicates and standard deviation minus background.

To obtain a broader view of the SpotMix™ PLUS performance, we screened healthy donors for different Epstein-Barr Virus (EBV) antigens and compared the data to standard PepMixes™ (Figure 5).

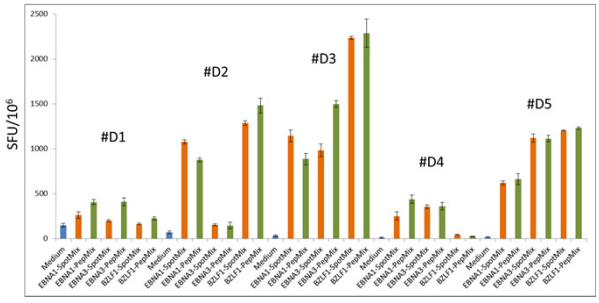

Figure 5: **Screening of 5 donors for T-cell responses against 3 EBV antigens using ex-vivo ELISpot.** Shown are the results comparing SpotMixes™ PLUS versus PepMixes™. Bars represent mean values of triplicates (and standard deviation) expressed as spot forming units (SFU) per Million cells.

Summary

Comparisons between T-cell stimulation assays performed with the classic PepMix™ peptide pools and SpotMix™ PLUS pools demonstrate equivalence of these preparations. We envisage that these novel pools will significantly facilitate T-cell protein target discovery by permitting the synthesis of protein-spanning, overlapping peptide pools for many potential target antigens in parallel.

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