The Source for All Peptides for Your Research

Make our Custom Peptides the Building Blocks of Your Success

Because of our very large and diverse synthesis capabilities and our research-only focus, **thinkpeptides** can offer you an exceptional package of price, quality, choice, and flexibility, with timely delivery anywhere in the world.

Whether you require milligrams or grams, crude or >98% pure, 1 or 100,000 peptides, or complex modifications, we'll always offer you a great deal.

Our custom peptide synthesis service offers a complete range of scales, purities and modifications. Peptides are suitable for many key applications, including antisera production, cellular assays, ELISA, ELISpot and crystallography.

Custom Peptide Synthesis Product Range

If you are using peptides for immunological studies, you will know the importance of having a product with full quality control, delivered quickly and at a market-leading price. Furthermore, you will want a supplier with the capability to offer you a large range of purities, quantities and specialist modifications to meet your needs. thinkpeptides (a brand of ProImmune) provides a full range of custom peptide products, peptide libraries and peptide microarrays, suitable for immunological applications such as T and B cell epitope mapping, T cell stimulation and production of antibodies. The service offers consistency of quality and value for long-term peptide requirements.

thinkpeptides custom peptide synthesis service is tailored to the needs of immunologists, setting us apart from other commercial peptide suppliers that just provide a basic service. Our highly qualified technical support team are ready to assist with issues such as peptide library design, product handling and storage. They can also advise on the design of experiments, such as ELISpot and intracellular cytokine staining.

Many of our customers require large numbers of peptides for their immunological applications and thinkpeptides is able to offer particularly competitive pricing for projects of this sort. We have expanded our range of peptide purities and quantities, offering even more choice and flexibility for our customers' experimental needs. Contact us for a special quotation tailored to your requirements. Confidentiality is maintained at all times.

Custom peptides undergo stringent quality control. MALDI-TOF Mass Spectrometry verifies peptide composition and reversed-phase chromatography verifies the purity. Your peptides are supplied with a copy of the analytical HPLC chromatogram and MALDI-TOF MS analysis for your records. Custom peptides are generally dispatched within 2-3 weeks, depending on the quantity and purity requested.

Our standard peptide synthesis service includes the following purities and quantities of peptide. Contact us if you require lower grade purity or larger amounts, we can also supply in gram quantities.

Purity	Crude, >70%, >75%, >80%, >85%, >90%, >95%, >98%
Quantity	1-4 mg, 5-9 mg, 10-14 mg, 15-19 mg, 20-24 mg, 25-29 mg, 50-59 mg
Typical dispatch time	Crude, >70% and >75% purity, 2-3 weeks >80% to >98% purity, 3-4 weeks

In addition to the synthesis options listed, a wide range of N-terminal, C-terminal and internal modifications and non-standard amino acids is available. The table shows a selection of the most commonly requested. If your modification of interest is not in the list, contact us with your requirements.

N-terminal	Acetylation Biotin FITC Dansyl 7-Methoxycoumarin Acetic Acid Formylation Myristic Acid Palmitoylation
C-terminal	Amidation pNitroanilide 7-Amino 4-methyl coumaride
Internal	Biotin Phosphoserine Phosphothreonine Phosphotyrosine Acetylation
Non-standard residues	Ornithine Norleucine Norvaline Homoserine Homocysteine Penicillamine Cyclization D-amino acids N-methyl amino acids
Other modifications	Cyclization Glycosylation

FAM-labeled Peptides used for Epitope characterization in Fluorescence Polarization Assays

Arun Rishi, Professor at the John D. Dingell VA Medical Center, Wayne State University in Detroit, USA, has been using thinkpeptides custom peptides in his research to study functions of the apoptosis-associated protein CARP-1, with the ultimate aim of developing small-molecule compounds as cancer therapeutics.

Almost ten years ago, Professor Rishi identified CARP-1 (cell cycle and apoptosis regulatory protein-1, also known as CCAR1) as a novel mediator of chemotherapy as well as a retinoid-induced cell death signalling in a number of cancer cell types, including breast carcinoma. With such a promising target in his hands, Professor Rishi was keen to develop his findings into novel cancer treatment agents. He realised that binding partners were key to the activity of CARP-1, since depending on context, CARP-1 can either inhibit or promote the growth of cancer cells.

Work in the Rishi laboratory is now centred on characterizing the epitopes within CARP-1 capable of mediating binding to its interaction partners, and then seeking small-molecule mimetics of these binding partners which could potentially act as therapeutic compounds. Professor Rishi uses a sophisticated fluorescence polarization assay to screen libraries of small molecules for binding to fluorescently labeled peptides derived from CARP-1. The quality of the peptide he uses in these experiments is paramount, and so he turned to thinkpeptides when he was looking for a new and reliable supplier.

Professor Rishi commented, "I have been consistently impressed by the customer service I received from thinkpeptides, as well as the quality of their products. They pay great attention to detail regarding my shipping requirements, are very easy to talk to, and are always very professional".

Improving Virotherapy Against Tumor Targets

Antitumoral immune response by recruitment and expansion of dendritic cells in tumors infected with telomerase-dependent oncolytic viruses

Ramakrishna *et al* Cancer Research. 2009;69(4):1448-58. [PubMed ID: 19190348]

Virotherapy is a unique and attractive idea for tumor therapy, and has the potential to specifically target tumor cells. Many anti-cancer therapies meet the problem of tumor-mediated tolerance. Danger signals from viral replication at a tumor site have the potential to make the tumor ‘visible’ to immune surveillance. Successful virotherapy depends upon dendritic cells (DC) being present at the tumor site to present antigen to tumor-infiltrating T cells.

Working at Hannover Medical School in Germany, Ramakrishna *et al* used model antigens to investigate ways of improving virotherapy through triggering DC recruitment to tumors. They used custom peptides manufactured by thinkpeptides (a brand of ProImmune) for ELISpot and CTL assay experiments designed to validate their new approach.

The team set up mouse models for both ovalbumin (OVA) and haemagglutinin (HA) –expressing tumors by manipulating peptide-expressing tumor cell lines. They established that the conditionally replicating telomerase-deficient adenovirus hTert-Ad would replicate in these cells, and induced primary tumors by subcutaneous injection of their cell lines into mice. Macrophage inflammatory protein 1-alpha (MIP1α) and Fms-like tyrosine kinase-3 ligand (FLT3L) were chosen as a combination of cytokines known to attract (MIP1α) and expand (FLT3L) DC *in vivo*. They showed that adenovirus-driven expression of these cytokines in tumour graft cells promoted DC and T cell infiltration of the tumor, which they hoped would improve their therapy further.

To validate their virotherapy design, the researchers performed IFN-gamma ELISpot experiments using thinkpeptides’ purified peptides covering the HA and OVA epitope sequences. Their results show that infection of tumors with actively replicating virus was an efficient way of generating HA- and OVA-specific IFN-gamma-producing CD8+ T cells. Combining virotherapy and cytokine expression gave rise to an even greater number of epitope-specific CD8+ T cells (figure 1).

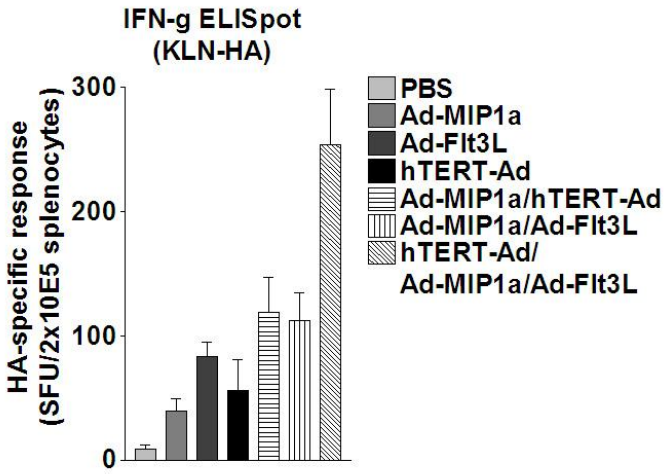


Figure 1: Immune response against intratumoral HA antigen measured by IFNγ ELISpot assays after incubation with MHC class I–restricted HA peptide. Data show responses measured from mice treated with cytokine-expressing adenovirus only, with both cytokines in combination, with replicating model-antigen expressing adenovirus alone, and then with the cytokines and replicating virus in combination, as labeled.

As a further test of the oncolytic capabilities of the CD8+ T cells from their virus-infected and tumor-bearing mice, custom peptides were used in an *in vivo* CTL assay. DC were introduced at the tumor sites to further enhance responses in these mice. Target cells were labeled *ex vivo* with the membrane dye CFSE and pulsed with OVA peptide, and then returned to treated mice via *i.v.* injection. Loss of the CFSE signal indicates that the target cells have been lyzed, demonstrating antigen-specific cytoxic activity (for example data, see figure 2). The best antigen-specific response was found in mice treated with replicating OVA-expressing adenovirus in combination with cytokines.

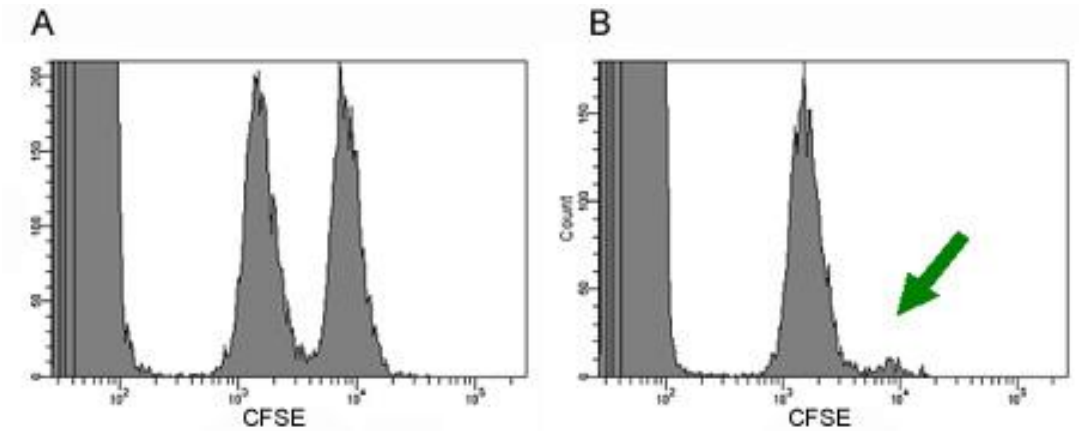


Figure 2. Systemic antitumoral immune response can be measured by CTL assay. Control peptide-pulsed target cells are loaded with a low concentration of CFSE (left peak), for comparison with target peptide pulsed cells, loaded with a higher concentration of CFSE (right peak). (A) results from untreated mice, and (B) results from tumor-bearing, treated mice. Target cells were lysed in the treated mice, as shown by the disappearance of the 'target' peak (green arrow).

In this study Ramakrishna *et al* have established a protocol for improving oncolytic virotherapy, using assays for evaluation of tumor response that relied on purified custom peptides from thinkpeptides. A sustained response directed against intratumoral antigens (such as the artificially expressed OVA and HA antigens used in this work) is highly desirable for cancer therapy, and the increased response that this team induced through stimulating DC recruitment and maturation makes this strategy even more attractive. It now remains to see if their approach can be successful in a clinical setting, against 'real' antigens.

This work was carried out at the Universities of Hannover, Germany, and Iowa, USA

Figures reproduced by kind permission of Norman Woller (Medizinische Hochschule Hannover)

Prospector™ Custom Peptide Libraries

Prospector™ custom peptide libraries allow the rapid screening of many peptides for bioreactivity. Overlapping peptide libraries can be used to investigate immune responses in detail and provide an opportunity to screen the immunological responsiveness of every optimal-length epitope in a given protein. They can be applied in a number of immunological assays including ELISpot and ELISA. Additionally, by generating a peptide library to test variations of the most common version of a particular epitope, it is possible to determine how specific amino acid mutations affect T and B cell or substrate recognition.

To meet the huge demand generated by the large number of applications for peptide libraries, we have significantly increased our peptide library product range:

Prospector™ Peptide Library Product Range

Code	Description	Purity	Quantity	QC	Estimated Dispatch
PP97	Prospector™ Pure97	>97%	>1 mg	100% LC-MS	Enquire
PP95	Prospector™ Pure95	>95%	>1 mg	100% LC-MS	3-4 weeks <200
PP90	Prospector™ Pure90	>90%	>1 mg	100% LC-MS	3-4 weeks <200
PP80	Prospector™ Pure80	>80%	>1 mg	100% LC-MS	3-4 weeks <500
PP70	Prospector™ Pure70	>70%	1-5 mg	100% LC-MS	3-4 weeks <500
PPLC	Prospector™ LCMS	Crude	1-5 mg	100% LC-MS	2-3 weeks <500
P0L	Prospector PEPscreen®	Crude	0.5-2 mg	100% MS or 100% LC-MS	2-3 weeks <500
PPLX	Prospector™ Lightning-X Peptides with C-terminal amino acid of choice	Crude	50-100 nmol	5% LC-MS	2-3 weeks <10,000
PPLG	Prospector™ Lightning-G Peptides with C-term. Gly	Crude	50-100 nmol	5% LC-MS	2-3 weeks <10,000

Prospector™ Pure

Our Prospector™ Pure purified peptide libraries deliver on the most exacting requirements and at prices that help you break new ground on research driven by synthetic peptides. Unlike the synthesis of individual purified peptides, these libraries are made using a parallel synthesis platform that guarantees minimal product variance, and delivery is in an easy to handle tube-array format. Our high throughput purification combined with 100% LC-MS analysis ensures that you do not have to lose critical path time on your projects just because you need to work with purified and fully analyzed peptides. Our technology also has the flexibility to meet multiple design parameters, such as N- and C-terminal modifications and non-standard amino acids.

Prospector™

Prospector™ libraries deliver highly attractive prices and exacting 100% LC-MS quality control (QC). Though these libraries are not purified, they are synthesized using the same reliable platform as our purified products and the resulting peptides have high average purity. For example, for Prospector PEPscreen®, the average purity of 10mers is ~86% and the average purity of 15mers is ~73%. The features of Prospector™ make it an ideal product for epitope discovery applications, giving you the comfort of comprehensive QC so you know absolutely what you are working with.

Unlike some commercial peptide libraries available, Prospector™ has no hidden set-up charges. A single price is charged per peptide, and the order size starts at only 24 or 48 peptides. Prospector PEPscreen®: Custom Peptide Libraries accommodate peptides from 6-20 amino acids; information in the table below about all other Prospector™ libraries is based on peptides from 7 to 15 amino acids; peptides up to 40 amino acids in length can be made using the technology platform, but quantities and dispatch times will vary, depending on the specification.

Technical support from thinkpeptides' experts

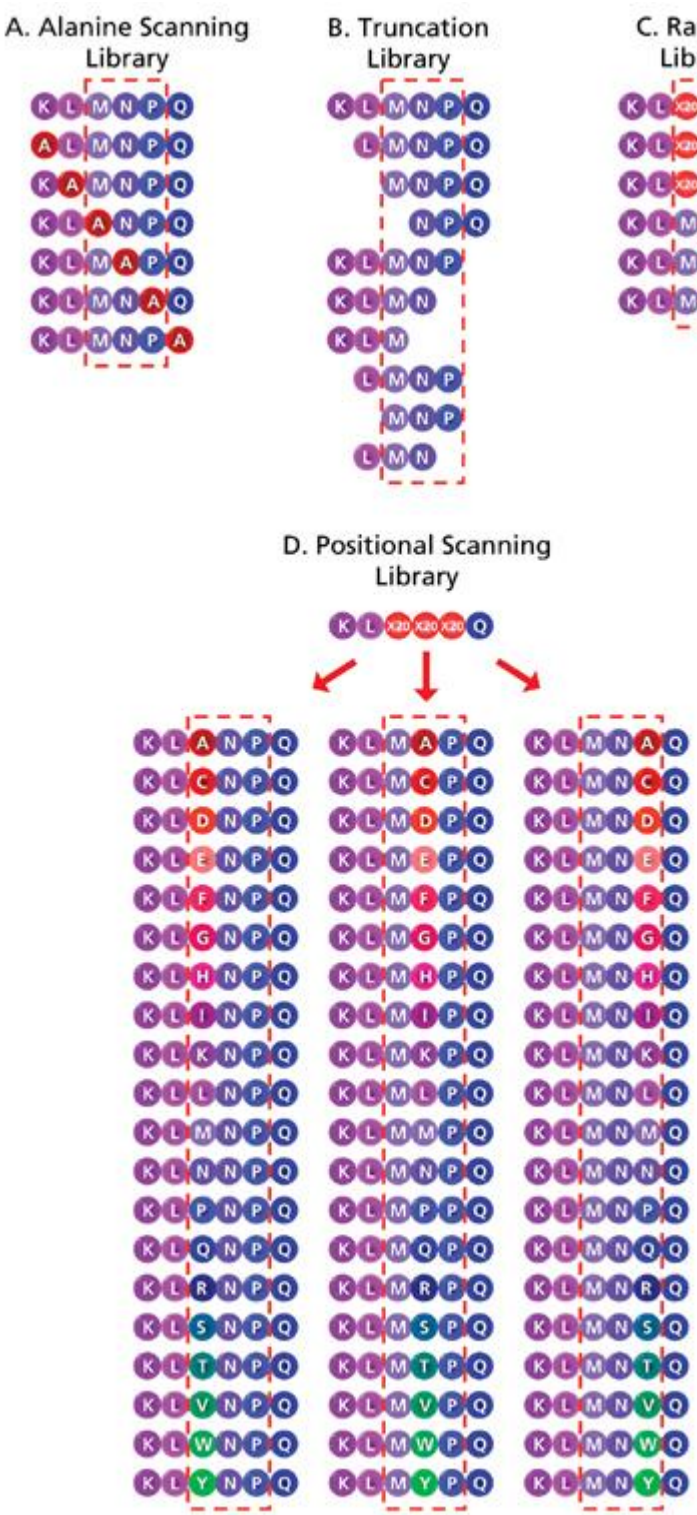
Our technical and applications support team can offer advice on library design, peptide solubilization, experimental set-up, and analysis. Relating to design, we can advise on the best overlap or offset of peptides to be used in your target application, and then using your full-length protein sequence, we can generate the list of peptides for your library. Assistance with experimental set-up and analysis is available for applications such as ELISpot, ELISA, cell culture, immunization protocols and flow cytometry. Additionally, ProImmune offers outsourcing of cellular analysis services, such as ELISpot and intracellular cytokine staining. The Prospector™ peptide library technology can be used for a number of different types of library, including overlapping peptide library, alanine scanning library, truncation library, random library and positional scanning library

Overlapping Peptide Library

The overlapping peptide library is most commonly used for linear, continuous epitope mapping, where the aim is to generate a library of overlapping peptide sequences of specific length and specific offset, to cover the entire native protein sequence. Choice of the appropriate peptide length and offset number depends on the application of the peptides and also affects both the cost of the peptide set and the usefulness of the data obtained from the experiment. Examples of design parameters for selected applications are shown in the following table:

Application	Length	Offset
ELISpot		
$CD4^+$ and $CD8^+$ T cell epitope mapping	15-20	1-5
$CD8^+$ T cell epitope mapping	8-15	1-5
MHC-Peptide Binding Assays		
$CD4^+$ T cell epitope mapping	15	1-3
$CD8^+$ T cell epitope mapping	9-10	1
B cell epitope mapping	15-20	5-10

Epitope identification is followed by studies to demonstrate structure and function relationships of peptide sequences, usually by peptide sequence optimization and structure stabilization. Synthesis of alternative types of peptide library can greatly assist the sequence optimization process. The figure shows a schematic representation of the different strategies in constructing peptide libraries for sequence optimization.



A. Alanine Scanning Library
Alanine is systematically substituted into each amino acid position in the identified epitope. This strategy identifies the amino acids in the native sequence that are essential for activity. Substitution of an essential amino acid results in a reduction in peptide activity, and the degree of reduction in activity is usually taken as a relative measure of the importance of the amino acid being substituted.

B. Truncation Library
This strategy determines the minimum length required for optimum peptide activity by generating a set of peptides with systematic truncation of the flanking residues. If the essential amino acids are known, the direction of truncation can be selected around them, as opposed to systematic truncation from both ends of the peptide sequence.

C. Random Library
Selected residues in the peptide sequence (wobbles) are simultaneously substituted with a mixture of all 20 amino acids, or a mixture of specific amino acids. In practise, this strategy is usually used for preliminary identification of a group of active sequences that can then be re-synthesized to validate the initial results.

D. Positional Scanning Library
A selected position or positions in a peptide sequence are each systematically replaced with different amino acids in order to determine the preferred amino acid residues at these positions, measured by corresponding increases in activity.

Figure 3: Schematic representation of the different strategies in constructing peptide libraries for sequence optimization. The presumed essential positions are enclosed in the dotted box.

Peptide Library Solubilization

Complete solubilization of peptides is important for successful screening of peptide activities. Peptides can be fully active only if they are completely solubilized and are able to assume the correct conformation for binding to their receptors. As the number of peptides in a set increases, so does the potential solubility variation of the peptides within the set. Therefore, in order to obtain accurate and reliable peptide activity data, careful attention should be devoted to the process of dissolving peptide sets.

The strategy for dissolving the PEPscreen® peptide set, and any of the other Prospector™ Libraries, is different from dissolving individual peptides. For individual peptides, conditions are chosen for optimum solubility based on the given peptide sequence. However, for peptide sets, conditions are chosen in an effort to dissolve as many of the peptides in the set as possible in the first solubilization attempt. Suggested common strategies are schematically represented in figure 4.

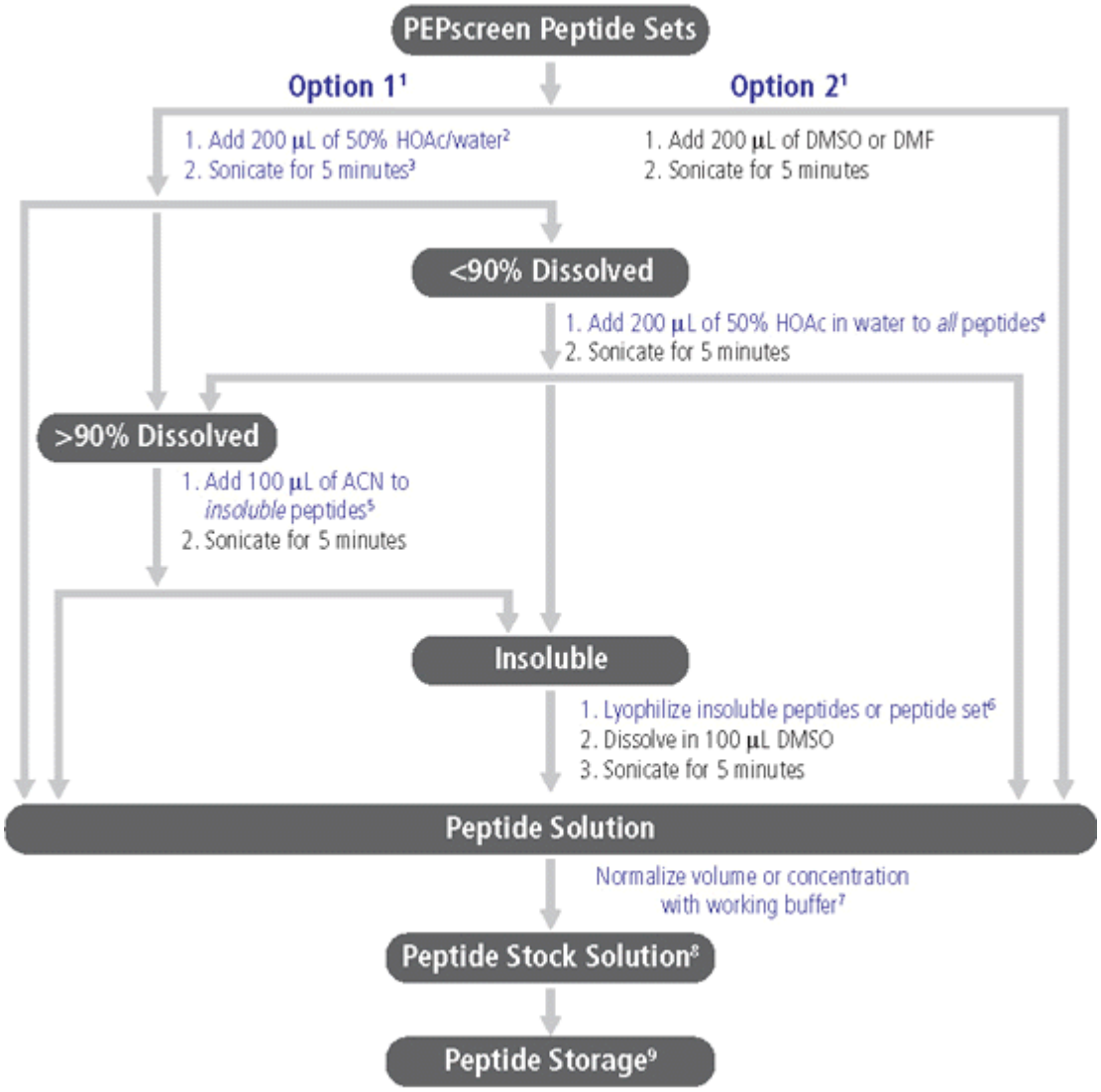


Figure 4: Suggested strategies for peptide library solubilization.

Quality Control

The power of high throughput LC-MS analysis

LC-MS means analysis by combined sequential liquid chromatography and mass spectrometry. Liquid chromatography is used to determine different species in each peptide and what proportion each species has overall. Mass spectrometry determines the molecular mass of a species and indicates by implication the molecular composition of a species. LC-MS quality control determines the mass spec of the major peak found by liquid chromatography. Combining the two methods means that it is possible to determine with certainty that the purified peptide is the species of interest. Prospector™ library peptides that fail quality control will be remade once. If a peptide still does not reach its specification the customer will be contacted to discuss options for re-synthesis.

For the Prospector PEPscreen® product, MALDI-TOF Mass Spectrometry (MS) is performed on 100% of samples. Each peptide must meet both the MS analysis and the final gross weight criteria to pass quality control. For a peptide to pass the MS criterion the desired molecular mass must be one of the three major ions. Peptides that fail quality control by MS will be remade once. Subsequent failed peptides are supplied as part of the order, and are labeled accordingly in the accompanying paperwork, allowing the user to decide whether or not to include the peptide in their studies.

Product Format

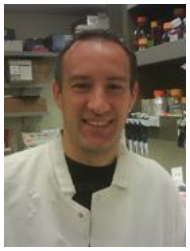
Prospector™ Pure, Prospector™ LCMS and Prospector PEPscreen®

Peptides are dried as a thin film at the bottom of individual tubes. This is to prevent the peptide from smearing throughout the inside of the tube during transit, which can make resuspension in small volumes difficult. Tubes are individually capped and arranged in a standard 8 x 12 tube array for compatibility with high throughput assays. This format also allows the flexibility to select only the tubes of interest and rearrange them into a convenient assay format. Each tube is clearly labeled in case the tubes are accidentally mixed.

Prospector™ Lightning

The peptides are dried as a thin film in a 96-well plate, with clear labeling for peptide set identification.

Research into Type I Diabetes therapy makes use of PEPscreen® technology



Bresson *et al.* (2010). Genetic-induced variations in the GAD65 T-cell repertoire governs efficacy of anti-CD3/GAD65 combination therapy in new-onset type I diabetes.

Mol. Ther. 2:307-316 [PubMedID: 19690518]

Photo: Dr. Damien Bresson, La Jolla Institute for Allergy and Immunology, USA

Bresson *et al.* investigated a novel combined therapy for type I diabetes consisting of treatment with anti-CD3 antibody plus a DNA vaccination with islet auto-antigen glutamic acid decarboxylase 65 (hGAD65). Mice from a C57BL/6 background and a NOD background were treated with either the combination therapy or individual anti-CD3 or GAD65 DNA vaccination.

GAD65-induced regulatory T cells (Tregs), splenocytes and pancreatic lymph node cells from treated mice underwent an *in vitro* proliferation assay using a PEPscreen® library of overlapping peptides from the hGAD65 sequence as a stimulant. The peptides had been pooled into 11 groups of 5 or 6 individual peptides. Combination therapy with anti-CD3/GAD65 markedly increased proliferation with 2 of the 11 peptide pools and to a lesser extent with a further 4 peptide pools when compared with individual therapies. The peptide pools that showed the highest response levels included previously described MHC class II immunodominant GAD65 epitopes (p27398-430 and p35524-543). These increased responses were only observed in the mice from a C57BL/6 background, correlating with an increase in GAD65 specific Tregs, whilst no differences between the combination and mono-therapies were observed in mice from a NOD background. This indicates that Treg responses to this combined treatment may be dependent on the MHC complexes expressed in an individual, highlighting the requirement to assess potential therapeutic functionality on a variety of genetic backgrounds.

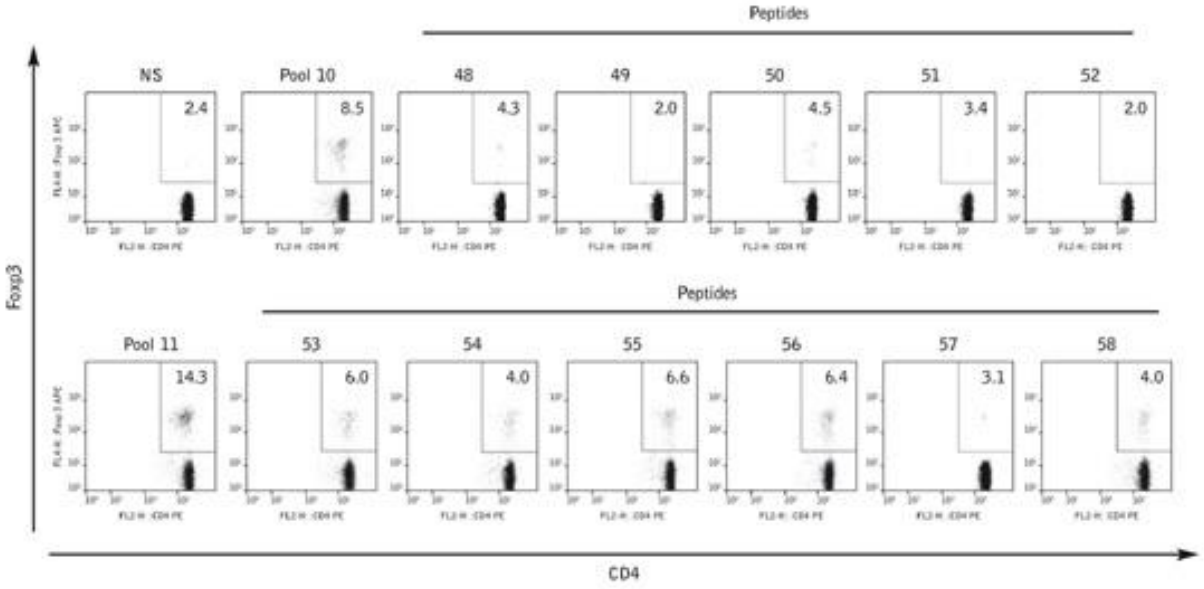


Figure 5: Glutamic acid decarboxylase of 65 kDa (GAD65)-specific regulatory T cells (Tregs) expanded in the RIP-LCMV-GP mice after combination therapy (CT) primarily recognize the C-terminal region of human GAD65 (hGAD65). Splenocytes and pancreatic lymph node cells were derived from NOD- and RIP-LCMV-GP-protected mice 4 weeks after treatment with NM-anti-CD3 alone or in combination with pCMV/ hGAD65 (CT). Splenocytes from RIP-LCMV-GP mice protected upon CT were stimulated with two pools of peptides (pools 10 and 11) or the single peptide from the pools 10 and 11 (covering the sequence 471–541 of hGAD65). On day 5 after stimulation, the percentage of Tregs was acquired by flow cytometry. Numbers shown in each histogram correspond to the percentage of Foxp3+ in the CD4+ T-cell population. Data are representative of four independent experiments. GP, glycoprotein; LCMV, lymphocytic choriomeningitis virus; NOD, nonobese diabetic; RIP, rat insulin promoter.

PEPscreen® used in vaccine development for Mycobacterium tuberculosis

(1) Roupie *et al.* (2008). Immunogenicity and protective efficacy of DNA vaccines encoding MAP0586c and MAP4308c of *Mycobacterium avium* subsp. paratuberculosis secretome. Vaccine 26:4783-4794 [PubMedID: 18652866]
(2) Romano, Rindi *et al.* (2008). Immunogenicity and protective efficacy of tuberculosis subunit vaccines expressing PPE44 (Rv2770c). Vaccine 26:6053-6063 [PubMedID; 18822333]

Mycobacterium avium subsp. paratuberculosis (MAP) protein is an etiological agent in chronic enteritis in ruminants, causing widespread disease. Development of a vaccine would aid some of the financial losses suffered by the livestock industry as a result of this disease. Roupie *et al.*(1), generated a PEPscreen® custom peptide library to screen overlapping peptides from two candidate antigens from the MAP protein (MAP0586c and MAP4308c) to identify immunodominant T cell epitopes that could be used as a potential vaccine.

Splenocytes from Balb/c and C57BL/6 mice immunized with plasmid DNA vaccines to the two MAP antigens were stimulated in vitro with PEPscreen® peptides and the levels of IL-2 and IFN gamma that were produced were measured by sandwich ELISA. For both antigens, stronger IL-2 and IFN gamma responses were seen for Balb/c mice than for C57BL/6. The results identified novel immunodominant T cell epitopes for the MAP0586c protein in MAP infected mice, but concluded that the weaker responses observed with the MAP4308c protein indicates that it is not an immunodominant T cell antigen in MAP infected mice.

In a further study by the same group, Romano *et al.*(2) used a PEPscreen® peptide library as part of their research into new potential subunit vaccines against tuberculosis (TB) in humans. An overlapping library for the PPE44 protein family of *M. tubercuolosis* was synthesized. There is little functional information currently available about the PPE proteins, but they are known to elicit strong immune responses in TB infected humans and mice. This study sought to map the specific responding peptides. As with the study by Roupie *et al.*, splenocytes from vaccinated and infected mice were stimulated with the overlapping PPE44 peptides and IL-2 and IFN gamma production was measured by ELISA. These experiments identified a strong potential CD4+ T cell epitope in the first 20 amino acids at the N-terminus of the protein. This appears to be a conserved area in a number of proteins in the PPE sub-family that elicit strong CD4 responses, which could be included in new subunit TB vaccines.

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