Comprehensive B Cell Epitope Prediction and Linear Mapping Services

ProImmune’s new REVEAL™ B Cell Epitope Discovery System offers rapid and cost effective epitope prediction and linear mapping of B cell epitopes for any application. Whether you are developing biological therapies or trying to understand diseases at a structural level, our services can help to accelerate your work, support the development of improved research hypotheses and develop robust, scaleable assays for measuring antibody responses.

**B Cell Epitope Prediction:** Predicts and ranks B cell epitopes in a protein of interest, based on the primary sequence and the three-dimensional structure, when available.

**B Cell Linear Epitope Mapping:** Mapping of the B cell linear epitopes in any protein or polypeptide sequence using antibody or antisera samples submitted by the client.

**ProArray™ High-Throughput Peptide Microarrays**

**New HLA-DP Alleles for REVEAL™ HLA-Peptide Binding Assays**

**Intracellular Cytokine Staining Outsourcing Service**

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**Customer Research**

**Angela Panoskaltsis-Mortari et al., University of Minnesota, Minneapolis, USA**

Pro5® Pentamers detect allospecific B cells by immunohistochemistry

**Samantha Westrop et al., Imperial College London, UK**

REVEAL & ProVE® Rapid Epitope Discovery System used to identify novel T cell epitopes in HIV-1

**Patrizia Stoitzner et al., Malaghan Institute of Medical Research, Wellington, New Zealand**

Study with Pro5® Pentamer demonstrates effective immunization method for whole protein vaccines
REVEAL™ B Cell Epitope Prediction Service

Our intelligent consensus prediction methodology uses a large collection of up to date public domain and commercially licensed scientific databases and software. A best practise approach has been developed to enable combination of the output from several different methods to generate the best possible consensus prediction.

The consensus prediction based on primary sequence combines a number of methods, including public and proprietary algorithms that evaluate:

- Antigenicity
- Hydrophobicity
- Hydrophilicity
- Flexibility
- Secondary structure prediction
- Beta-turn structure analysis
- Protein physiochemical properties
- Protein physiochemical properties

These algorithms and their interpretation are applied to primary protein sequences regardless of whether or not a three dimensional structure is available. On request, the top consensus predicted epitopes could be mapped to protein sequences available in public databases and to protease cleavage sites for the protein of interest for analysis of potential degradation.

Where a three dimensional structure is available it can be used to evaluate the characteristics as described above but in addition we can provide the degree of actual epitope surface exposure and association with beta turn structures. This information is used to improve the relative ranking of predicted epitopes.

Case Study: Correlation between ProImmune’s B cell epitope prediction service and published epitopes for Human Papillomavirus (HPV) type 16 major late capsid protein L1

The HPV L1 protein is the main immunogen used in Merck & Co.’s Gardasil® vaccine. Gardasil® is a quadrivalent prophylactic vaccine produced against the most prevalent types of HPV – types 6, 11, 16, and 18. Gardasil® contains recombinant L1 for each of these HPV types adsorbed to aluminium and as such it is one of still only a few recombinant sub-unit vaccines currently licensed by the FDA. HPV 16 and 18 alone are responsible for 70% of cervical cancer cases and also cause a range of other cancer types. HPV 6 and 11 cause about 90% of genital warts cases. Cervical cancer is an important public health problem in the United States, with 9710 new cervical cancer cases and 3700 deaths due to cervical cancer estimated for 2006. The disease is caused almost exclusively by unresolved HPV infection.

Exploring the nature of the antibody epitopes of the L1 protein would help in understanding the function of the L1 protein as an effective immunogen in more detail, and would yield information that could be applied to the design of further recombinant sub-unit vaccines.
Figure 2: Analysis of HPV 16 L1 – conceptual workflow.
To determine the effectiveness of ProImmune's epitope prediction, an extensive literature search was done to identify published epitopes for HPV 16 L1. These epitopes were compared with epitopes predicted by ProImmune.

Figure 3: Crystal structure of HPV16 L1 protein with the predicted epitopes mapped onto the surface. Colors are used to distinguish adjacent predicted epitopes.

Figure 4: Blue underlined = epitopes predicted by ProImmune; bold blue underlined = epitopes recommended by ProImmune for antibody production; bold green = extensions to epitope region recommended by ProImmune for antibody production; red underlined = epitopes predicted in the literature.

Ten epitopes published in the literature overlapped at least to some extent with predicted epitopes. In seven out of nine cases (78%), predicted sequences overlapped substantially with published epitopes. Two out of fifteen predicted epitopes could not be verified in the published literature.
REVEAL™ B Cell Linear Epitope Mapping Service

The REVEAL™ Rapid B Cell Linear Epitope Mapping Service provides mapping of the B cell linear epitopes of a protein or polypeptide sequence using human serum or plasma samples submitted by the client.

Mapping linear epitopes can be done quickly with well-defined, easily controllable, high throughput technologies, which include standard rapid synthesis of overlapping peptide libraries and standard immunoassays. Although not all conformational epitopes may be mapped, linear B cell epitope mapping can be a quick way of getting a relative ranking of antigenic regions within proteins and between different proteins in a group.

Using the REVEAL™ epitope mapping service the specific linear protein segments from any larger protein or peptide that bind to antibodies in a given sample, such as sera or plasma, can be identified. In this way, 'hot-spot' areas of the protein can be discovered. Alternatively, if these areas of the protein sequence are already defined, the optimal epitope sequence can be elucidated using REVEAL™ with a peptide library with an offset of one, or with a truncated peptide library, or with a series of peptides with single amino acid substitutions.

Applications of the B Cell Linear Epitope Service:
- Definition and ranking of individual linear epitopes in an antigen
- Identification of peptides for immunization
- Discovery of cross-reactive determinants between related proteins
- Definition of strongly antigenic regions in proteins
- Comparison of different serovars or subtypes in terms of their relevant antigenicity
- Combining linear epitope mapping with epitope prediction

The REVEAL™ B Cell Linear Epitope Mapping Service uses standard immunoassay methodologies to screen plasma or sera samples submitted by the customer. Based on the proteins of interest, ProImmune can design and synthesize an appropriate peptide library for the project. Our services include cost-effective solutions for a wide range of project requirements, from a handful of peptides screened against a similar number of antibody samples, to hundreds or even thousands of peptides screened against hundreds of antisera using ProArray™ Custom Peptide Microarrays.

ProArray™ High-Throughput Peptide Microarrays

Our new ProArray™ peptide microarrays deliver a truly game-changing advancement in protein science. Drawing on the scalability of DNA microarray technology, peptide arrays deliver speed throughput and consistency in proteomics, which has traditionally been hampered by sluggish and highly manual laboratory techniques.

ProArray™ delivers between fifty and tens of thousands of peptides immobilized in array format on glass slides. ProArrays™ are printed in three identical sub-arrays with high intra-chip reproducibility. The glass slides can be incubated with a single 500μl volume sample. This minimizes sample use and greatly simplifies measuring a large number of interactions simultaneously.

Figure 5: Conceptual Layout of a ProArray™.

![Figure 5: Conceptual Layout of a ProArray™](image-url)
Peptides are first synthesized as a peptide library and subsequently printed on standard reader compatible microarray glass slides in triplicate sub-arrays. The chemoselective immobilization process ensures that only full-length peptides are attached to the slide via the N-terminus. Peptide arrays are faster to make and are more cost effective than whole protein arrays. The chemical synthesis method ensures a higher batch-to-batch reproducibility, leading to better validation and standardization for use in immune monitoring assays in clinical trials. Additionally, ProArrays™ can accommodate peptides with modifications such as phosphorylation, or non-natural amino acids such as citrulline, and are more stable than protein arrays, allowing storage over a longer period.

ProArray™ peptide library microarrays are ideal for high throughput sequence mapping of protein-protein interactions, including mapping of antibody epitopes, receptor ligand interaction studies, and profiling major enzyme classes such as kinases, phosphatases, and proteases. The technology allows you to map interaction hotspots in a series of proteins, or study an entire viral genome at once, and correlate outcome with reactivity, whether for a single time-point or in a longitudinal study.

Up to 100,000 peptides can currently be spotted on a ProArray™, which means that even if you are studying dozens of proteins you can get fast, consistent results for all of them screened against a single sample. Additionally, 200 copies of the same array can be printed from a single peptide library, enabling a study of up to 200 donors in a cohort in a single screening project. Our process delivers unrivalled flexibility, throughput and value.

The ProArray™ technology allows whole proteomes to be spotted onto slides, which the user can then screen with donor samples from different disease states for the systematic identification of peptide biomarkers. Smaller, focused arrays can then be made for the hits from the proteome screening process, for immune monitoring purposes or research into antibody therapeutics or vaccines.

Case Study: Pro5® Pentamers detect allospecific B cells by immunohistochemistry

Graft rejection following bone marrow transplantation is a serious problem for patients who have undergone this treatment. Amongst other factors, the presence of preformed alloantibody is a major contributor to the failure of bone marrow engraftment.

A recent study by Panoskaltsis-Mortari et al. demonstrated a novel protocol using ProImmune’s Pro5® MHC Class I Pentamers to detect allospecific B cells in situ by immunohistochemistry. Splenocytes from BALB/c mice (H2d) were injected into C57BL/6 mice (H2b) that were then sacrificed after 3 weeks. Splenic cryosections were stained with an H-2Kd-specific Pentamer (GYKDGNEYI) and, as a negative control, an H-2Dk Pentamer (RRLGRTLLL). BALB/c H-2Kd specific cells were detected in the splenocyte sections, whilst there was no binding of the H-2Dk Pentamer in serial sections. As an additional control C57BL/6 mice were injected with B10.BR splenocytes (H2k). In splenic cryosections the H-2Dk Pentamer showed specific staining, whereas there was no staining with the H-2Kd Pentamer.

This is the first study using Pro5® Pentamers to detect allospecific B cells by immunohistochemistry. This could be an important technique to provide further information about spatial positioning of alloantibody producing cells and inter-cellular interactions. This method could also be used as a way of visualizing antigen specific T cell responses in tissues.
Figure 6: Allospecific B cells can be detected in situ in allosensitized murine spleen using Pro5® Pentamers. Colors for the different markers are indicated in colored text. Magnification is 400x (left and middle panels) and 200x (right panels). Copyright American Society of Hematology.
New HLA-DP Alleles for REVEAL™ HLA-Peptide Binding Assays

To overcome the inadequacies of publicly available class II HLA epitope prediction algorithms, ProImmune has developed a comprehensive, high throughput, in vitro class II HLA-peptide binding assay and a complementary assay to determine HLA-peptide binding stability. The assays now include 20 HLA-DP alleles, in addition to the 56 DR and DQ alleles previously available. HLA-DP is known to have a clear relevance in many disease settings, but less is understood about it relative to HLA-DR and DQ. The availability of HLA-DP alleles as part of the REVEAL™ assays will contribute to the investigation of HLA-DP-peptide binding and shed light on how the allele influences disease susceptibility and resistance. The new expanded range of more than 70 alleles covers nearly all of the major ethnic groups worldwide. ProImmune's REVEAL™ class II HLA binding and stability assays are the only such comprehensive assays offered or even described by any group anywhere.

The REVEAL™ HLA-peptide binding assays are the initial step in the REVEAL & ProVE® Rapid Epitope Discovery System, which analyzes proteins for class I and class II T cell epitopes. They also perform a critical role in the REVEAL™ Immunogenicity System, which predicts the antigenicity of drug candidates for the purpose of lead optimization. The HLA-peptide binding assays determine the exact binding sequence and HLA restriction of a potential T cell epitope in a single step. Our cell-free, in vitro assays are rapid and do not consume precious cell samples. Subsequently, the class I binding peptides identified can be confirmed using MHC Class I ProVE® Pentamers in flow cytometry, and the putative class II epitopes can be validated using functional cellular assays, such as T cell proliferation assays and ELISPOT. These highly sensitive methods can demonstrate whether a particular peptide sequence can actually cause a significant T cell response in samples from a cohort of donors.

### Alleles available for REVEAL™ class II HLA-peptide binding and stability assays

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### Alleles available for REVEAL™ MHC-peptide binding and rate assays

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Case Study: REVEAL & ProVE® Rapid Epitope Discovery System used to identify novel T cell epitopes in HIV-1


The genetic profile of an individual, such as the patient’s tissue type, is understood to greatly influence the rate of HIV-1 disease progression. For example, HLA-B35 and -B8 have been associated with an increased rate of disease progression in HIV-1 individuals. ProImmune’s REVEAL & ProVE® technology was used to investigate the HIV-1 Gag-specific CD8+ T cell immune response mounted by HLA-B35+ individuals to discover the possible reasons for this rapid advancement in the disease.

Westrop et al. used the REVEAL™ MHC-peptide binding assay to screen 9-mer peptide sequences from the HIV-1 Gag protein against the HLA-B*3501 allele. A previously unknown epitope was identified, which may be involved in an immune response in B*3501-positive individuals.

ProImmune synthesized a PEPscreen®: Custom Peptide Library of forty-four 9-mer HIV-1 Gag peptides. These peptides were assessed for binding to B*3501 using the REVEAL™ assay. The binding affinity of the peptides was compared to a pass/fail control peptide known to have marginal affinity for the B*3501 MHC complex. Peptides with a conformational signal >80% of the pass/fail control signal were considered to be potential epitopes (Figure 7). Twelve HIV-1 Gag peptides fulfilled this criterion, and these peptides were further analyzed using the REVEAL™ Quick Check Off Rate Assay. The rates of dissociation of the peptide-MHC complexes were measured at 0, 2 and 24 hours and the half-lives of the complexes were calculated to give an indication of their overall stability. Two peptides, YPLTSLRSL (YL9) and HPVHAGPIA (HA9) were found to form a stable complex with B*3501, indicating that these may be potential novel epitopes.

Figure 7: Results of the REVEAL™ MHC-peptide binding assay for controls and the 12 peptides that were considered potential epitopes; binding signal is shown as a percentage relative to the pass/fail control; peptide 8, HPVHAGPIA (red) was further validated by Pro5® Pentamer staining.

Westrop et al. then carried out Pro5® MHC Class I Pentamer staining, which showed a well-defined Pentamer+/CD8+ population for the B*3501/HPVHAGPIA epitope (figure 8). Additionally, no naïve or central memory T cells specific for the HA9 peptide were found, indicating a lack of proliferative potential in infected HIV-1+ individuals. Further validation with ELISPOT assays showed that the HA9 HPVHAGPIA peptide elicited the highest ex vivo response of the peptides studied.

Figure 8: Pro5® Pentamer staining of live lymphocytes gated on CD3+ cells; the left plot shows staining with an allele mismatched negative control Pentamer, the right plot shows staining with the HA9 (B*3501/HPVHAGPIA) Pentamer. 0.39% of CD8+ live lymphocytes are CDB+/HA Pentamer+ with a background stain of 0.03%.

The author commented that this study “confirms the novel ProImmune technology as a useful and superior tool for revealing potential immunogenic epitopes”, and also highlighted the importance of validating results in parallel using functional assays, such as ELISPOT and Pro5® Pentamer staining.
Case Study: Pro5® Pentamer demonstrates effective immunization method for whole protein vaccines


Immunotherapy using dendritic cells (DC) loaded with tumor antigenic peptides has been shown to lead to improved survival and tumor regression in cancer patients. Epicutaneous immunization through the skin is a new and interesting approach to deliver antigen to resident skin DC. However, the delivery method of these vaccines should be improved to enhance cytotoxic responses.

In this study by Stoitzner et al., epicutaneous immunization was carried out on mice using the ovalbumin (OVA) protein administered in a cream via the ear skin. The immunizations were given in the skin using protocols to induce migration of skin DC. The method of epicutaneous immunization targets epidermal Langerhans cells as well as dermal DC as antigen presenting cells, both of which can present the vaccine effectively to the immune system.

An OVA specific Pro5® MHC Class I Pentamer (H-2Kb/SIINFEKL) was used to detect antigen-specific CD8+ T cell responses by flow cytometry in red-cell lysed tail vein blood. The levels of CD8+ Pentamer+ cells were increased nearly four-fold in mice immunized through barrier-disrupted skin compared to normal skin and a PBS-immunized control. This T cell response remained higher for up to 21 days after immunization.

Skin disruption would be an easy and non-invasive method to use in clinical trials. To date this method of delivery has been used mainly with single peptide vaccines. This study highlights that the epicutaneous immunization method can be used with a whole protein antigen. Whole protein vaccines could be given to patients with any HLA allele and could elicit an MHC class II response as well as a class I response.
Outsource Your Intracellular Cytokine Staining

ProImmune offers rapid and reliable flow cytometric detection of IFN-gamma production in CD4+ and CD8+ T cells through its experienced applications team. By outsourcing your intracellular cytokine staining experiments to ProImmune you take advantage of our technical proficiency and save time by passing collection and analysis of data to our experts.

Intracellular cytokine staining (ICS) can be used as an immune monitoring tool to measure the immune response to known antigens, or for epitope discovery, combined with ProImmune’s REVEAL & ProVE® technology, to identify and/or validate novel T cell epitopes. Flow cytometric detection of intracellular cytokines allows for simultaneous phenotyping and gating of cells, to select the specific live lymphocyte population.

Sample Shipping and Handling

All cellular analysis services are carried out at ProImmune’s qualified facilities in the UK. We have substantial experience in the shipping of customer cell samples from overseas, including from the USA and Australasia. We handle the shipping process seamlessly from your facility through our own pre-qualified, specialized sample shipping providers. Alternatively you can use your own shipper under our instruction.

For each project we will discuss any special requirements for sample handling with you in detail in advance. When shipping, we ensure that all required customs and export regulation information is on hand, and in the unlikely event of any delay in the package clearing customs, we and our shippers ensure that dry ice is topped up to maintain sample temperature.

ProImmune’s cellular analysis services offer you rapid and affordable T cell assays run to optimized protocols. We currently offer:

- Intracellular cytokine staining for IFN-gamma
- ELISPOT assays for IFN-gamma and IL-2
- Flow cytometric detection of antigen-specific T cells with ProVE® or Pro5® MHC Class I Pentamers
- T cell proliferation assays with peptides or whole antigens
- HLA tissue typing of donors

All our services are designed to help you accelerate your immune monitoring or epitope discovery projects by saving you the cost and effort of setting up and maintaining these platforms in your own laboratory. Contact us for a quotation tailored to your needs.