PROIMMUNE
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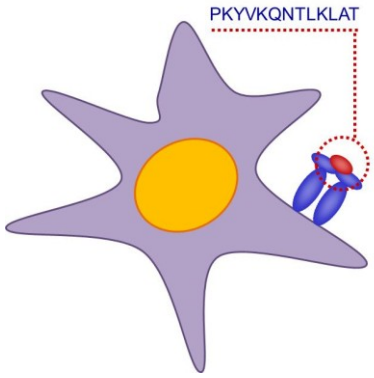
ProPresent™ Antigen Presentation Assay

Master Immunogenicity, Accelerate Product Development



- Identify naturally presented peptides directly
- Determine which portions of your drug, vaccine or compound are visible to the immune system
- Compare proteins, protein formulations and the effect of donor HLA types
- Compatible with fully-formulated biologics

The ProPresent™ Antigen Presentation Assay tells you exactly which epitopes from your biotherapeutic drug or other protein of interest are presented by HLA Class II molecules to T cells. It is the only commercial service that determines the peptides that are actually presented by dendritic cells (DC) following uptake and processing of your protein. Peptides are identified by the classical method of HLA-peptide complex extraction, peptide elution and subsequent peptide epitope identification by sequencing mass spectrometry. Combined with ProImmune's REVEAL™ HLA-peptide binding assays and functional T cell assays, ProPresent completes the picture in understanding the immunogenicity of your compounds.

Knowledge gained from ProPresent™ and ProImmune's REVEAL™ Immunogenicity services provides you with the information needed to understand and manage immunogenicity of biotherapeutics or other protein compounds. Our system permits comparison of data from a set of donor samples and can help explain whether patients with particular HLA-types could be at higher or lower risk of an adverse reaction to a biological compound. The assay is compatible with fully formulated biologics and can also be used to compare different proteins, or different formulations of the same protein for an alteration in the pattern of presented epitopes. ProImmune's whole protein DC-T cell and peptide T cell proliferation assays can be employed to confirm functional relevance of the epitopes identified by ProPresent™.

Applications for ProPresent™

- Epitope discovery and characterization
  - Establish naturally processed epitopes for monitoring a patient response to a vaccine or biologic
  - Profile known or suspected allergens
  - Identify the immunological impact of sequence variants of the same protein, e.g. in viral proteins, or for tumorigenic point mutations
  - Investigate the population bias of epitope responses using HLA-typed donors

- Risk Assessment of Biologics
    - Identify presented epitopes in food additives or other consumer goods, e.g. cosmetics
    - Identify presented epitopes in pharmaceuticals and other biological products
    - Compare and contrast the presented epitopes from different batches, formulations, or production methods for the same biologic, to pre-empt safety concerns
- Profile the responses to biosimilars and biobetters
- Establish a baseline for safety assessment; compare novel agents to established, safe, comparator proteins
- Generate data to support a regulatory submission

Process Flow for ProPresent™

- Protein is supplied by the customer
- A panel of e.g. 10 HLA-typed, healthy donor peripheral blood mononuclear cell (PBMC) samples are prepared from the ProImmune tissue bank (selected to reflect HLA distribution of choice)
- Monocytes from donor PBMC are cultured in defined media and differentiated to produce dendritic cells (DC)
- DC are loaded with the test antigen and induced to mature
- DC are harvested, HLA-DR molecules are purified and the associated peptides are eluted
- Peptide samples are analyzed by sequencing mass spectrometry
- The mass spectrometry data is compared against a protein database library consisting of the sequence of interest and the international protein index (IPI) of the organism of choice
- Peptides are ranked by significance according to a probability based algorithm
- Data is verified by searching against a scrambled decoy database to reduce false positives
- A full data report is compiled, listing all detected epitopes including presentation of nested epitope sets and anchor analysis against well-known HLA-DR alleles with a summary of detected control proteins.
- Delivery time can be approximately 6 weeks, depending on the scope of the project

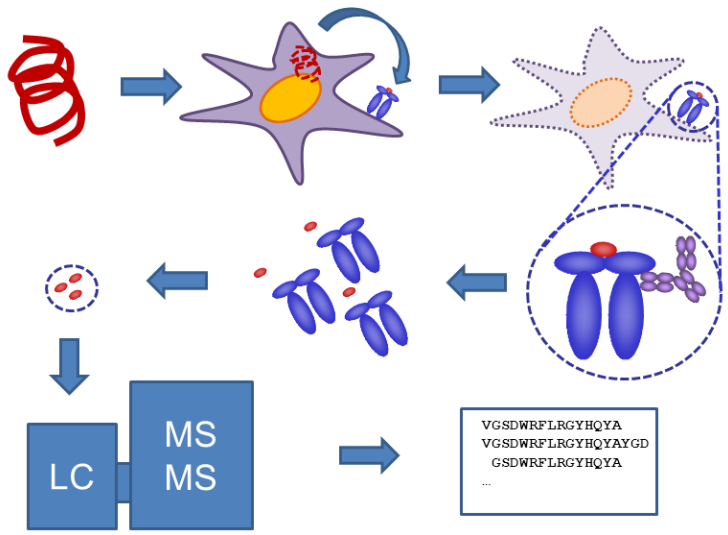


Figure 1: ProPresent workflow. The protein of interest is cultured with dendritic cells (DC). DC take up the protein and process it. HLA Class II molecules present epitopes from the protein on the DC surface.

Cells are lyzed and HLA Class II-peptide molecules are then recovered in an immune affinity step. Peptides are recovered from the HLA molecules and analyzed by sequencing mass spectrometry.

Peptides identified by mass spectrometry are subjected to rigorous analysis to identify true positive peptides with high confidence.

ProPresent™ in Context

ProImmune provides several modular tools for understanding immune responses. The *in vitro* methods we offer can define sequences within an antigen that can bind to HLA molecules, and whether or not these sequences cause T cell responses. Functional assays however do not reflect the many complex internal cellular processes important in the presentation of antigens by HLA Class II. These processes are accounted for using the ProPresent™ Antigen Presentation Assay, which determines the repertoire of naturally presented peptides in antigen-pulsed DC. The methodology automatically includes natural editing activities, such as protease-based cleavage and peptide editing by HLA-DM and other antigen presentation pathway components.

The following table summarizes key elements that form part of an ideal immunogenicity risk assessment strategy for a biological compound and which of ProImmune's services is most appropriate for each stage.

	ProImmune REVEAL HLA-peptide Binding Assay	ProImmune Naïve T cell Assay	ProImmune DC-T cell Assay	ProPresent Antigen Presentation Assay
Natural Antigen Processing	X	X	✓	✓
Assessment of peptide binding to MHC	✓	X	X	✓
T cell functionality	X	✓	✓	X
Epitope Identification	✓	✓	X	✓

A strategy for early determination of immunogenicity risk for biotherapeutic proteins is of immense value in the drug development cycle. The use of a combination of technologies to assess and reduce immunogenicity liabilities at the lead optimization stage will result in improved programs and acceleration of products to market.

ProPresent™ Data

Humira® (Adalimumab) is an established anti-human TNFα (human tumor necrosis factor alpha) therapeutic monoclonal antibody used in the treatment of conditions such as Crohn's disease, rheumatoid arthritis and ankylosing spondylitis. 5-20% of patients repeatedly dosed with Humira® produce anti-drug antibodies, so ProPresent™ was applied to find potentially immunogenic regions.

20 donors of a range of HLA types chosen to cover the HLA-DR expression distribution in the global population were tested in the assay.

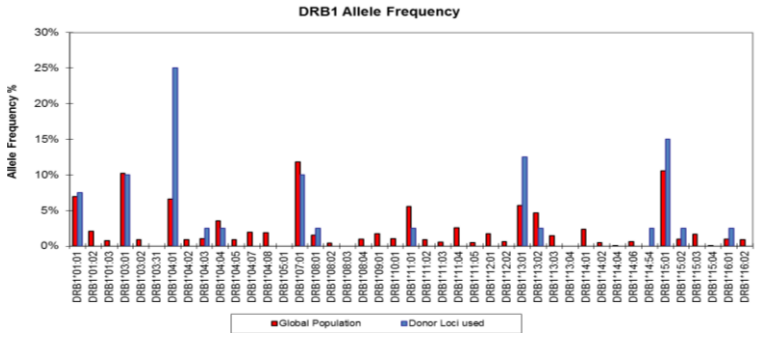


Figure 2: HLA-DR expression patterns in the global population (red) and donor cohort (blue) used in ProPresent™.

From a 122 amino acid residue stretch of the Humira® variable heavy chain sequence (full sequence information for Humira® is not available in the public domain), 3 potential epitopes were identified using ProPresent™, including one (DNAKNSLYLYLQMNSLRAEDTA) which was presented by a range of donors of different HLA-DR types.

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RDNAKNSLYLQMNSLRAEDTA*
-DNAKNSLYLQMNSLRAEDT*
-DNAKNSLYLQMNSLRAEDTA*
---AKNSLYLQMNSLRAEDT*
---AKNSLYLQMNSLRAEDT*
---AKNSLYLQMNSLRAEDTA*
---AKNSLYLQMNSLRAEDTA*
---AKNSLYLQMNSLRAEDTAV*
----KNSLYLQMNSLRAEDT*
----KNSLYLQMNSLRAEDTA*
----KNSLYLQMNSLRAEDTAV*
-----NSLYLQMNSLRAED*
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------SLYLQMNSLRAEDT*
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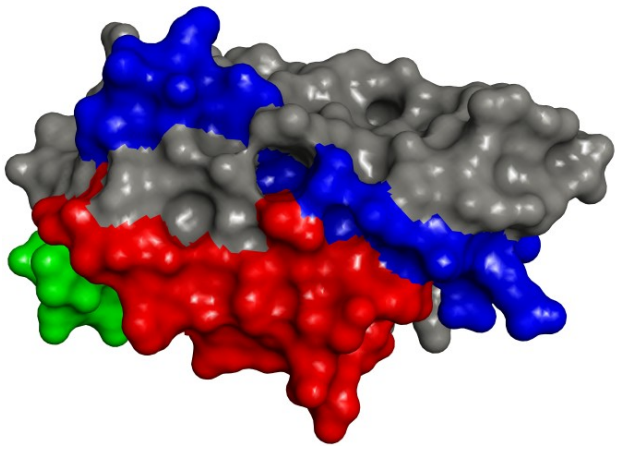

Figure 3: ProPresent™ data for Humira® variable heavy chain segment. Left panel shows one of the nested sequence sets identified by mass spectrometry, covering the putative DNAKNSLYLYLQMNSLRAEDTA epitope. Right panel shows the three epitopes identified for this region, mapped on to the structure of human IgG1k. DNAKNSLYLYLQMNSLRAEDTA is shown in blue.

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