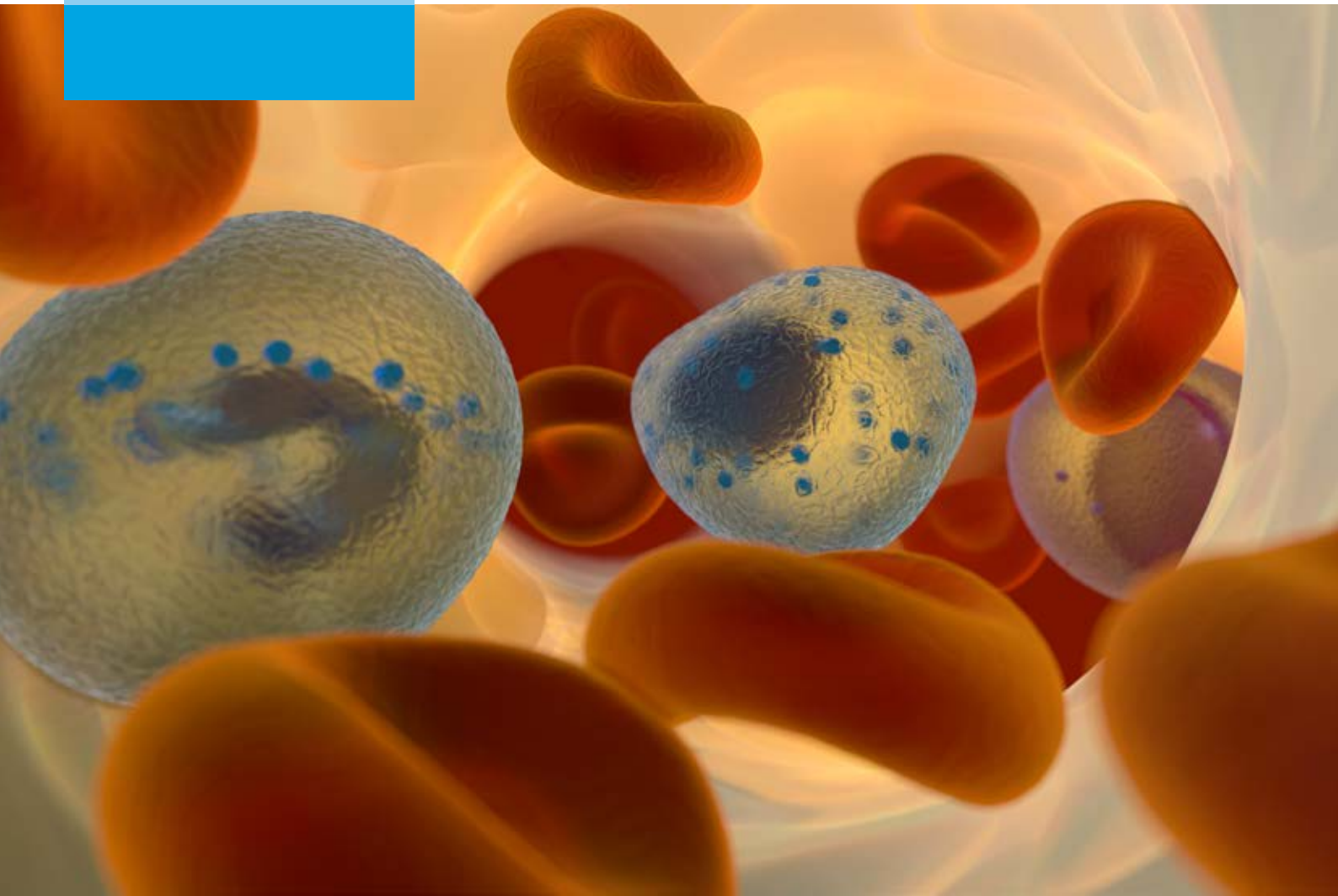


Immunogenicity Assessment Services
Improve Safety, Efficacy
and Cost of Development



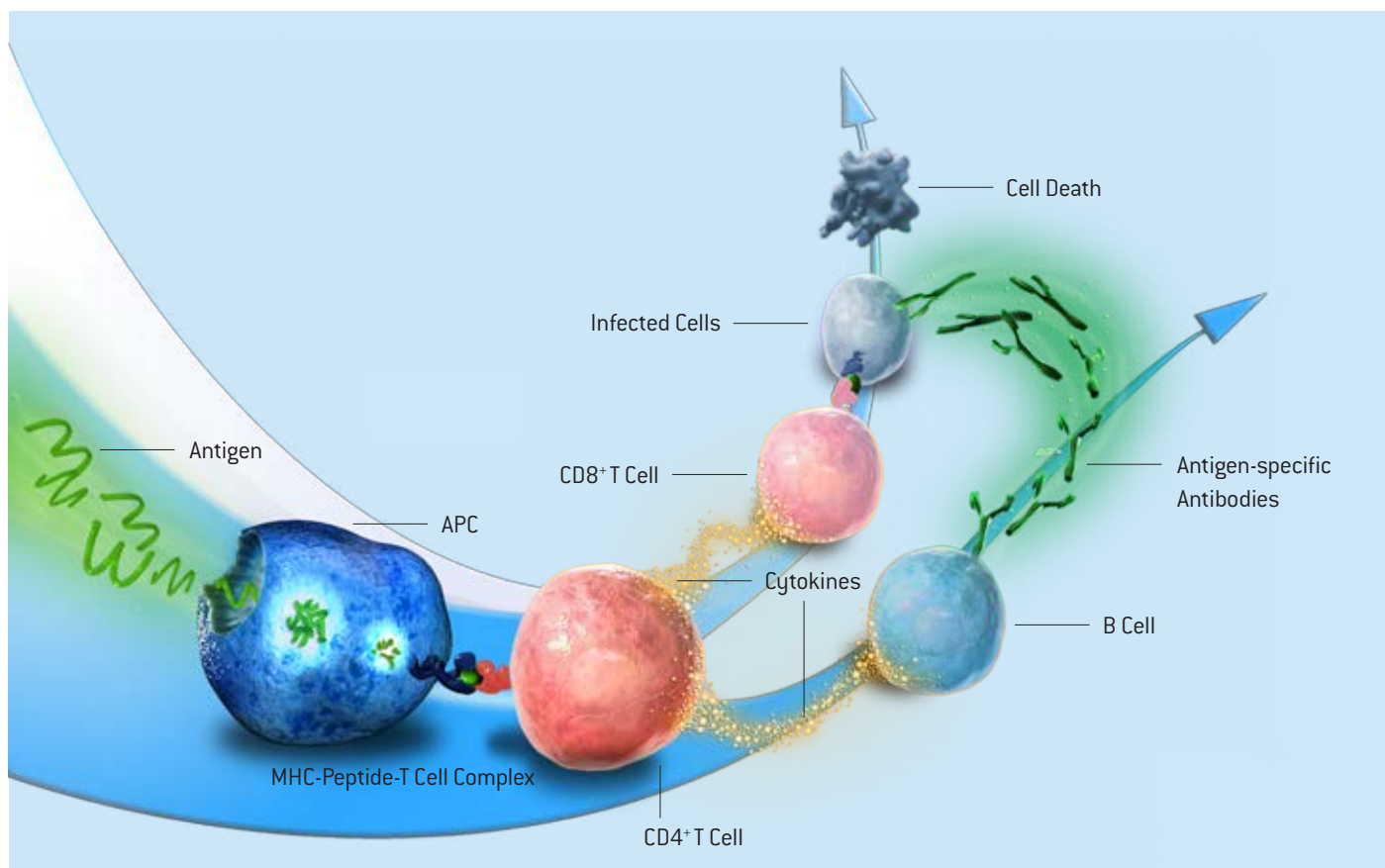
Immunogenicity Affects Candidate Success

The safety and efficacy of biological candidates are critical to their clinical success. The proteinaceous nature of many drug candidates can lead to an immunogenic response in the patient. The proteins may aggregate or have epitopes that are recognized by the immune system. Once the immune system is activated, the efficacy of the biotherapeutic declines as the system attempts to clear the antigen. The immune response itself affects product safety as cytokine release can be toxic to the patient. For biotherapeutic development, avoiding this response is vital for success.

Alternatively, for vaccine development, triggering an immune response is desired. Designing and assessing vaccine candidates for the appropriate immune response is critical for their success.

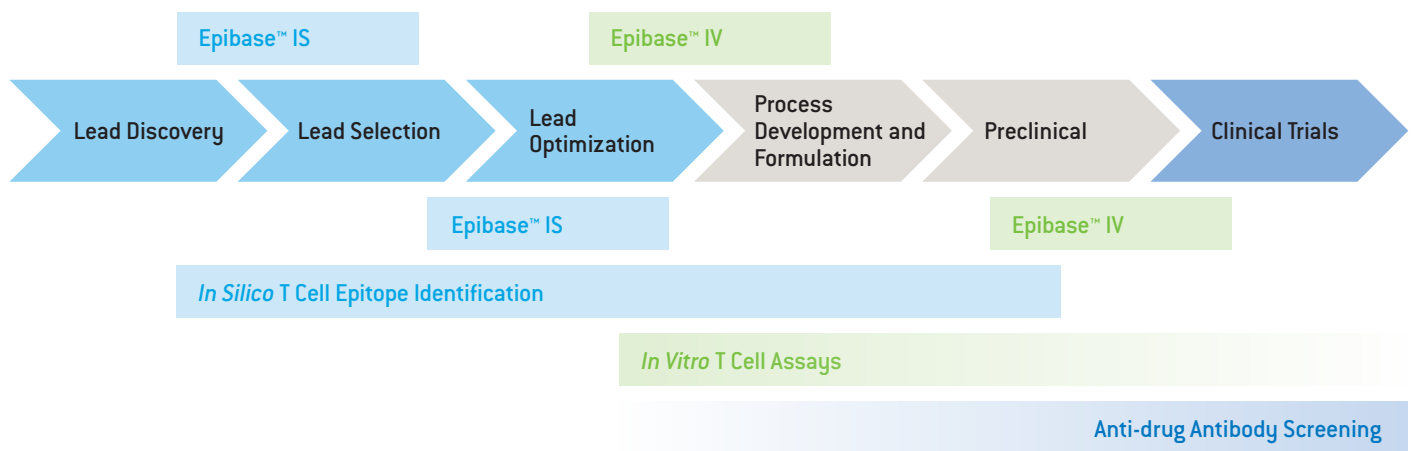
Animal testing is used in an effort to assess the potential immunogenicity of biotherapeutic and vaccine candidates. The response in animals can be helpful to researchers; however, it is often not a good indicator of what the human immune response will be to the biological candidate.

An antigenic response to the therapeutic candidate initiates T and B cell responses that may affect efficacy and safety.



Lonza Tools Predict Immunogenicity

Lonza offers *in silico* and *in vitro* assessment services to screen biotherapeutic and vaccine candidates for their immunogenicity potential. Our Epibase™ tools help predict the immunogenicity of candidates promoting knowledge-based decisions regarding candidate selection in the early discovery and development phases. The tools can also aid in the monitoring of immunogenicity during clinical phases.



Epibase™ *In Silico* Epitope Screening Platform

The Epibase™ *In Silico* (IS) tool is a patented, industry-leading T cell epitope screening platform that analyzes and predicts the potential immunogenicity of proteins. Epibase™ IS is a predictive tool driven by sequence information and structural bioinformatics in conjunction with experimental human leukocyte antigen (HLA) binding data. The tool uses structural characteristics of the HLA receptor along with experimentally determined binding affinities to help determine the likelihood of peptide/HLA binding, a condition necessary for T cell activation. The use of structural information enhances the accuracy of the predictions when compared to approaches based on sequence alignment alone.

For biotherapeutics, Epibase™ IS addresses immunogenicity in a global population, with over 99% coverage. Eighty-five (85) HLA Class II allotypes are represented encompassing the genetic variation in populations such as Caucasian, Asian, Hispanic, African American, and others. Immunogenicity can be predicted for a global population, or can be tailored to a specific population to which a biotherapeutic is targeted.

For vaccines, Epibase™ IS predicts the immunogenicity potential of candidate peptides or whole molecules based on HLA Class I and II epitopes. Thirty-five (35) HLA Class I and 85 Class II allotypes are represented. The IS tool can help optimize vaccine design.

Epibase™ *In Vitro* Screening Platform

Lonza offers the Epibase™ *In Vitro* (IV) immune screening platform that utilizes a high throughput, multi-parameter approach to analyze T and B cell responses to candidate biotherapeutics and vaccines. Analysis is conducted using multiple platforms including Flow Cytometry, ELISPOT and ELISA.

Cellular Immunoprofiling Provides the Following for Your Lead Candidates:

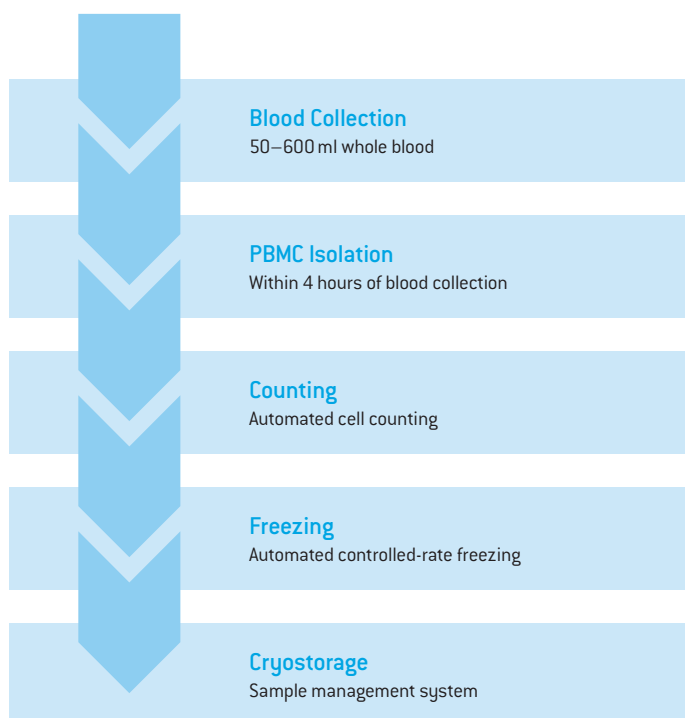
- Ranking and selection
- Comparison
- Optimization
- Surveillance

The Epibase™ IV assays use human peripheral blood mononuclear cells (PBMCs) providing researchers with an immune response that closely resembles the response in a human. IV assessment data supports requests from regulatory authorities for the inclusion of more human safety information in regulatory filings. The Epibase™ IV assays provide fast, cost-effective ways to gain human-based immunogenicity information on candidates prior to the first human dose.

Human Response with PBMCs

For the Epibase™ IV assays, whole blood is collected at clinical sites adhering to ethically approved protocols. PBMCs are isolated and cryopreserved within 6 hours of blood withdrawal. All PBMC preparations are genotyped to a 4-digit level at HLA Class I and II loci, and undergo stringent quality control analysis, including cell viability, functionality and sub-population analysis. The Lonza Applied Protein Services team holds a Human Tissue Authority license for the storage of isolated PBMCs for subsequent research use.

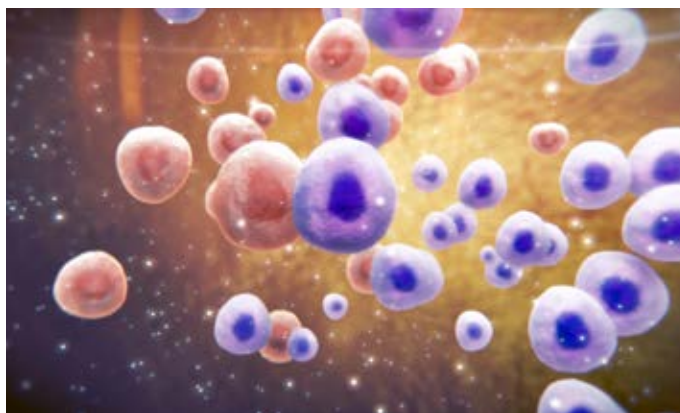
PBMCs can be isolated from healthy donors, who would form a typical donor panel. With access to over 200,000 donors, Lonza has the option to source donors from diseased populations, selected ethnicities or by gender and age in order to test biotherapeutics or vaccines in a donor population representative of the disease and patient target.



PBMCs are isolated from donated blood and cryopreserved within 6 hours according to our standard procedures.

Epibase™ IV T Cell Assays

Analysis of T cell responses is a frequently used tool to monitor the activation of the immune system. The assays take into account not just the molecule, but other factors which may contribute to immunogenicity such as production contaminants, aggregates and formulation components. Many Epibase™ IV assays can be performed on “whole” molecules or peptides to support the development of both biotherapeutics and vaccines.



Epibase™ *In Vitro* assays assess the immunogenicity potential of biological candidates by detecting the proliferation of T and B cells as well as cytokine release.

Proliferation Assays

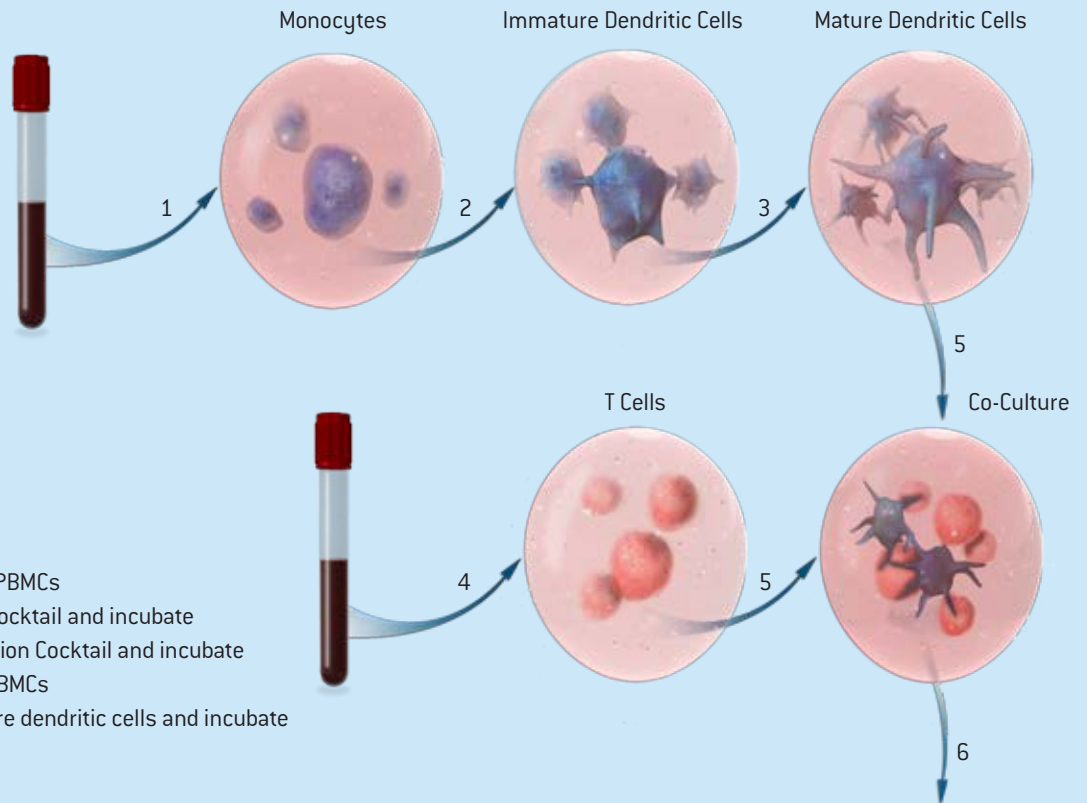
Flow Cytometry based detection of cell proliferation is used in conjunction with cell surface markers to provide a comprehensive description of T cell activation and proliferation status. Flexible assay formats allow for the screening of candidates with different modes of action. Candidates can be screened by addition to whole PBMCs with assessment of proliferation (indication of immune response) occurring after an optimized incubation period (Whole PBMC Assay). Alternatively, monocyte-derived dendritic cells can be incubated with the antigen and then co-cultured with autologous CD4⁺ T cells prior to measurement of cell proliferation (DC:CD4 Assay). Proliferation assays can be combined with other detection methods, such as cytokine profiling, for T cell monitoring.

Cytokine Assays

Cytokine analysis by Flow Cytometry or enzyme-linked immunosorbent spot (ELISPOT) assays measure the production and release of cytokines by individual T cells. These robust, versatile and accurate assays are widely used to monitor the immune response. Large panels of cytokines also can be profiled using high throughput multiplex methods.

In addition to the standard assays, our highly sensitive, enriched ELISPOT assay can be used for the identification of low frequency memory CD8⁺ T cell responses, which can be used to support rational vaccine design.

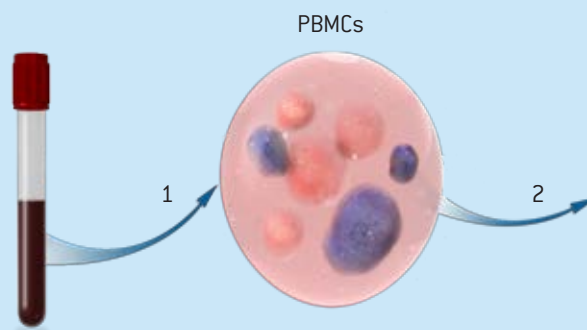
DC:CD4 Assay



Assay Steps Include

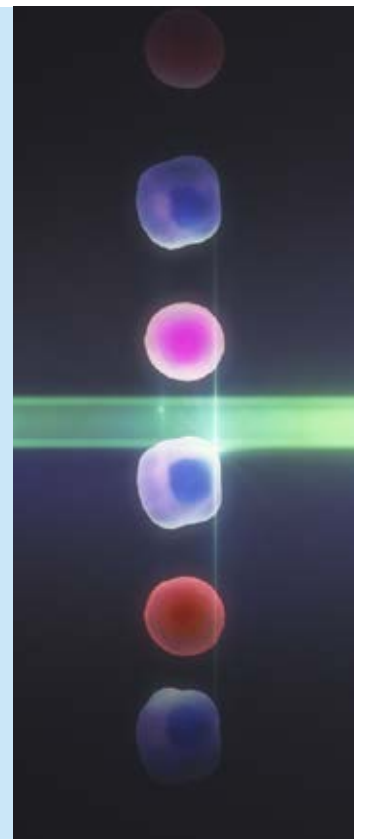
- 1 CD14 isolation from PBMCs
- 2 Add Differentiation Cocktail and incubate
- 3 Add antigen, Maturation Cocktail and incubate
- 4 CD4 isolation from PBMCs
- 5 Mix T cells and mature dendritic cells and incubate
- 6 Detection step

Whole PBMC Assay



Assay Steps Include

- 1 Add antigen to PBMCs and incubate
- 2 Detection step



Epibase™ IV B Cell Assays

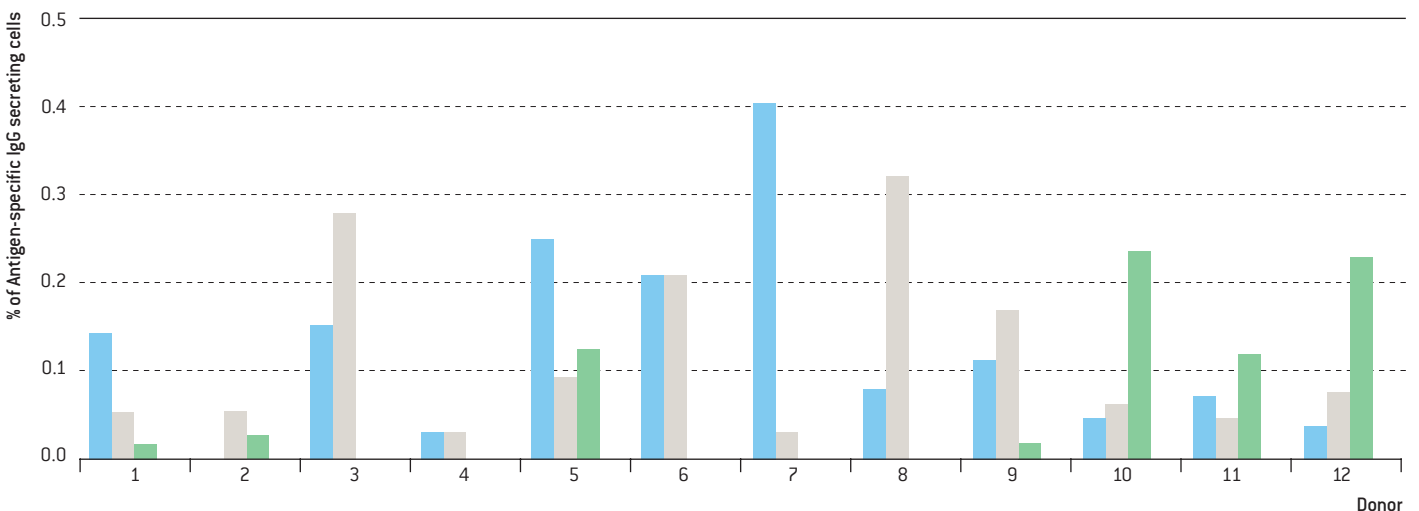
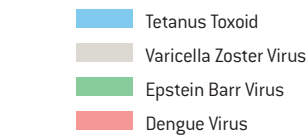
Characterization of *in vitro* B cell responses on a cellular level has become an effective tool to obtain antibody measurements. The B cell ELISPOT assay is a highly sensitive assay designed to quantitate antibody-secreting B cells. B cell assays can support knowledge-based vaccine design, including both adjuvant and antigen screening. Additionally, the assays can test for pre-existing humoral immune response, as well as monitor B cell responses during trials using biotherapeutics.

Recall B Cell ELISPOT Assay

The recall B cell assay utilizes ELISPOT technology for the detection of total and antigen-specific IgA, IgG, IgE, and IgM production in blood samples (PBMC) or suspensions of lymphoid tissues, enabling the identification and enumeration of total and antigen-specific secreting cells (ASCs). The ELISPOT assay is highly sensitive, allowing for the analysis at the single cell level and quantitation of the number of circulating antigen-specific memory B cells.

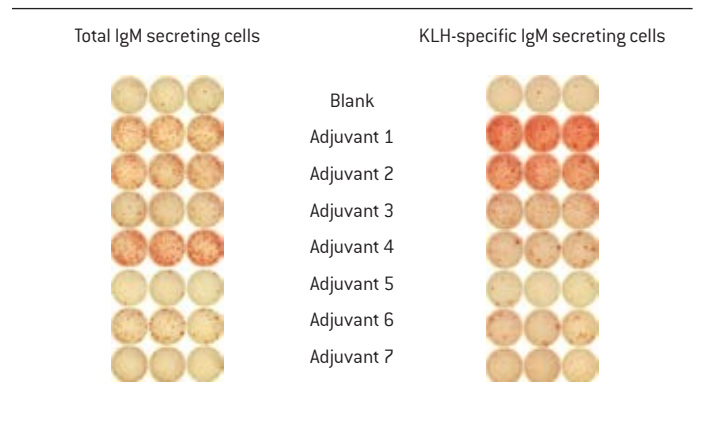
For biotherapeutics, the assay can be applied to the detection of pre-existing and/or therapy-induced antibodies (therapy surveillance) and as a potential antibody detection method for cases of strong interference of free drug in serum samples.

For vaccines, the assay can be used to detect B cell responses towards natural infections or after vaccination (recall responses), as represented in the graph below.



Adjuvant Assay for Vaccines

The adjuvant *in vitro* assay is unique to the vaccine field. It is used for the compatibility and response assessment of candidate and reference vaccine adjuvants in combination with the immunizing antigen. The assay can be used as pre-selection prior to or as an alternative to animal studies. The adjuvant assay uses the ELISPOT method for the detection of total and antigen-specific IgM production by donor cells at the single cell level. Results allow for the determination of the most potent adjuvant /antigen combination, based on the quantitative measurement of antibody secreting cells after *in vitro* immunization. The assay can be performed in combination with T cell analysis (proliferation, cytokine production, etc.) for analysis of both humoral and cellular immune responses.

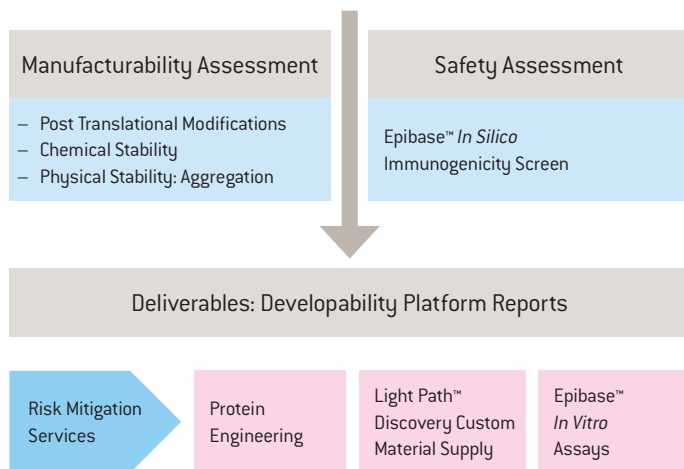


ELISPOT images of total (left) and antigen-specific (right) IgM producing cells after *in vitro* immunization with 7 test adjuvants and KLH peptide. Each spot represents an antibody-secreting cell.

% of Antigen-specific IgG secreting cells per total IgG secreting cells for 12 individual donors.

Partnering for Immunogenicity Assessment

The Lonza Applied Protein Services team can partner with you during discovery and development stages to help you predict the immunogenicity potential of candidate biotherapeutics and vaccines. In addition to the Epibase™ IS and IV assays, Lonza offers our Light Path™ small-scale non-GMP manufacturing service. The Light Path™ Discovery Service can provide mg quantities of your lead candidates for your *in vitro* assays and other development activities. Additionally, our Applied Protein Services team can work with you to develop custom assays to suit your individual project needs. This can include assay design and validation with subsequent transfer to your team or a transfer of your assay to Lonza.



Lonza's Applied Protein Services include the Developability Assessment Toolbox and follow up Risk Mitigation Services.

Epibase™ IS and IV Profiling Helps Maximize Clinical Success

The Epibase™ Screening platform provides immunogenicity data to help you assess your biotherapeutic and vaccine candidates. The Epibase™ IS epitope screening tool can support lead candidate identification in the early stages including immunogenicity prediction in a target population. The use of PBMCs in the *in vitro* assays predicts the human response and is the closest you can get to the human immune system prior to the first human dose. The Lonza Epibase™ IV platform allows lead candidates to be tested in healthy and/or diseased populations. *In vitro* testing allows for the determination of immunogenic capacity, comparison and ranking of different leads early in the development process.

When used in combination with our *in silico* tools, the Epibase™ IV assays can help guide the rational selection of the “best” candidates to go on to the clinic and de-risks the investment in subsequent clinical trials. Choosing candidates for further development based on knowledge of their immunogenicity potential, helps maximize R&D resources, reduce timeline and costs, and increase regulatory compliance.

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