RESEARCH. DELIVERED.

PBL ASSAY SERVICES
Understanding the role of protein biomarkers in various disease states is essential, as are the most robust tools for examining how your therapeutic candidates modulate these biomarkers. The roles and regulation of cytokines and interferons are crucial in many therapeutic indications, and new applications for these biomarkers continue to be identified as detection technology evolves. PBL's Assay Services capabilities can help you to better quantify the key biomarker proteins that impact your clinical development programs.
PBL Assay Science is a science-driven outsourcing partner that provides reliable, high quality work on-time and on-budget. We have built our reputation over 25 years in business by listening to our clients and delivering the products and services they demand. Our approach is to tailor the service to meet your project needs, not a one-size-fits-all attitude.

Your confidentiality is important to us. All services are provided under strict non-disclosure terms and conditions.

**Quantitative Immunoassays**
- Ultra-Sensitive fg/ml Cytokine & Biomarker Quantification Services
  - Quanterix Simoa®
  - Singulex Erenna®
- Single Analyte ELISAs
- Multiplex Screening Service

**Cell-Based Assay Services**
- Cytokine/Chemokine Bioactivity Assays
  - Proliferation & Anti-Proliferation
  - Cytokine Secretion
- Antiviral Assays
  - Cytopathic Effect Inhibition (CPE)
  - Neutralizing Antibody

**Reagent Development Services**
- Protein Production & Purification
- Antibody Production & Characterization
- Hybridoma & Cell Line Development
- Protein & Antibody Labeling

**Additional PBL Services**
- Assay Design & Development
- Assay Optimization
- Technology Transfer
- Life Science Consulting Services
PBL’s sample testing and screening services will expedite your research and development work. Our existing technologies and services can be tailored to meet your specific requirements under fit-for-purpose guidelines.

Whatever your sample testing and screening sensitivity needs, PBL can help you detect your analyte(s) of interest. From state-of-the-art technologies quantifying into the femtogram/milliliter level to simultaneous detection of up to 16 analytes in a single well, PBL is a partner you can trust.

Services include:

- Ultra-sensitive Biomarker & Cytokine Detection (fg/ml)
  - Quanterix Simoa®
  - Singulex Erenna®
- Single Analyte ELISA (pg/ml)
- Multiplex ELISA (up to 16 analytes)
Quanterix Simoa®

Ultra-Sensitive fg/ml Biomarker Quantification Services


Take your detection to the next level.

- Single Molecule Array technology delivers femtogram per milliliter (fg/ml) level sensitivity for measurement of low-abundance biomarkers in serum, plasma, and other matrices
- Unique technology provides robust detection for single analytes and select multiplex panel options
- High precision service offers accurate and reproducible data with <10% inter- and intra-assay CVs
- Over 40 analytes available including IL-17A, TNF-α, IL-23, TGF-β, TRAIL, IL-6, IL-10, IL-12p70, Aβ-40, Aβ-42, Tau, p-Tau231, and NF-LIGHT®

The unique immunoassay technology provided by the Simoa® platform† allows PBL to deliver fg/ml level sensitivity for low-abundance biomarkers in serum, plasma, and other matrices. Utilizing Single Molecule Array technology which traps a single bead in a femtoliter-sized well allows for “digital” measurement of each bead. This approach can increase assay sensitivities several orders of magnitude over conventional bioassays.

The combination of this instrument’s markedly heightened sensitivity and its capacity for digital analyte measurement ensures accuracy and precision across a wide dynamic range and minimizes sample volume requirements.

†Powered by Simoa® Technology, Quanterix Corporation

Unprecedented precision can be obtained using Simoa’s “digital” readings of single molecular events. In the image above, individual paramagnetic beads are trapped in femtoliter-sized wells, then sealed over and quantified by SIMOA image analysis.

Human Cytokine 3-Plex A Immunoassay Standard Curves
(AEB: Average Enzyme per Bead)

Normal human serum endogenous levels of several pro- and anti-inflammatory cytokines
Singulex Erenna®

Ultra-Sensitive fg/ml Cytokine Testing Services

**Accurate and Robust Measurement. Increase your Understanding.**

**Detect at unprecedented levels.**

- Cytokine analysis utilizing Single Molecule Counting (SMC) technology enables sub-pg/ml sensitivity for accurate measurement of low abundance biomarkers in normal or disease serum/plasma.
- Minimal sample dilution requirement with 4-Log+ dynamic range.
- High precision cytokine immunoassays provide reproducible data.
- Extensive analyte menu of over 35 different cytokine assays covering several disease areas including IFN-γ, IL-17A, IL-17F, IL-23, TNF-α, IL-6, and IL-10.

The ability to accurately measure low-abundance analytes present in complex matrices is essential for the profiling and characterization of cytokines and other biomarker analytes. Employing proprietary single-molecule counting (SMC) technology with robust microparticle-based Erenna® immunoassays††, PBL’s cytokine detection services can provide scientists with sub-pg/ml level measurements of low-abundance analytes in healthy or disease sera and plasma.

An increased understanding of the role and regulation of cytokines and interferons in disease states results from greater profiling and characterization of their activities in biological responses. Singulex technology can be instrumental in furthering our collective understanding of low-abundance cytokines in complex matrices.

††Powered by the Erenna® Immunoassay System, Singulex, Inc.

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**Technology Overview**

Low abundance biomarkers in complex biological samples are transferred to a 96-well plate and incubated with capture antibody-coated microparticles. After washing and incubating with dye-labeled detection antibodies, wells are again washed to remove unbound detection antibodies. An elution buffer is added, and the eluate drawn into the instrument for single-molecule counting.

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The scatterplot indicates IL-17F levels in human sera (n=20) and plasma (n=7). Median quantifiable levels of IL-17F are 0.67 pg/ml and 0.91 pg/ml in sera and plasma, respectively.

Median Heparin-Plasma (IL-1b) = 0.20 pg/ml
Median Citrate-Plasma (IL-1b) = 0.08 pg/ml
Median EDTA-Plasma (IL-1b) = 0.06 pg/ml

Overall Median (IL-1b) = 0.08 pg/ml
Single Analyte ELISA Service

**Sensitive Detection and Robust Results. Dedicated, Flexible Workforce. Reliable, High-Quality Outcomes.**

Take advantage of PBL ELISAs or other commercial ELISAs for a wide range of additional analytes using PBL’s services.

**PBL ELISAs:**
- High sensitivity ELISAs available for a comprehensive range of species and analytes
- Compatible with a variety of matrices including serum, plasma, tissue culture media (TCM), and cell lysates
- Matrix tolerance for autoimmune disease sera*
- Reproducible results with low intra- and inter-assay CVs

PBL ELISA kits are heterogeneous antibody sandwich immunoassays. The antigen is captured by an antibody immobilized to the wells in a microtiter plate and a secondary antibody is used to complete the sandwich. The detection system which utilizes horseradish peroxidase (HRP) and tetramethyl-benzidine (TMB) as the enzyme-substrate provides a sensitive and robust assay for the detection and quantification of interferons and cytokines.

PBL routinely runs ELISA studies for our clients for the detection and differentiation of interferons and cytokines in various sample matrices. We offer a range of heterogeneous immunoassay services for:

- **Human** IFN-Alpha*, Beta*, Gamma, Lambda 1/2/3, Omega, Gamma Receptor 1
- **Mouse** IFN-Alpha, Beta, Lambda 2/3
- **Cynomolgus/Rhesus** IFN-Alpha, Beta

**High Sensitivity Human IFN Alpha All Subtype ELISA**

IFN-alpha specific signal was measured in sera of Lupus patients. Blue bars represent non-specific signal, i.e. signal not blocked by Anti-IFN-alpha PAb.

**High Sensitivity Human IFN Beta Serum ELISA**

IFN Beta was measured in sera/plasma of Multiple Sclerosis (MS) patients and Normal Donors. IFN Beta was found to be quantifiable in 86% of MS patients on IFN Beta therapy, 4.1% of MS patients on other therapies, and 1.5% of Normal Donor samples.
Technology Overview

PBL’s multiplex ELISAs employ a technology in which distinct capture antibodies are individually spotted into each well of a 96-well ELISA plate in a defined array. These plates are run similar to a normal ELISA run. An image of the plate is then taken using a CCD camera.

Using image analysis software to perform subsequent analysis, the chemiluminescent results for each analyte allow for a deeper understanding of the cytokine profiles of the samples.

<table>
<thead>
<tr>
<th>Category</th>
<th>Multiplex Technology</th>
<th>Electro-Chemiluminescent</th>
<th>Bead-Based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Data Output</td>
<td>1536 data points / test</td>
<td>864 data points / test</td>
<td>1000 data points / test</td>
</tr>
<tr>
<td>Sample Size</td>
<td>15-50 μl / test</td>
<td>50 - 100 μl / test</td>
<td>Difficulty with Serum and other viscous samples</td>
</tr>
<tr>
<td>Sample Type</td>
<td>Many Types</td>
<td>Many Types</td>
<td></td>
</tr>
<tr>
<td>Variation</td>
<td>Low Variability ≤ 7% CV</td>
<td>Acceptable Variability ≤ 10% CV</td>
<td>Bead-lot to lot variability</td>
</tr>
<tr>
<td>Cost per Data Point</td>
<td>&lt; $1.50 / data point</td>
<td>~ $2.00 / data point</td>
<td>~ $2.00 / data point</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Low pg/ml</td>
<td>Low pg/ml</td>
<td>Low pg/ml</td>
</tr>
</tbody>
</table>

PBL offers cost-effective multiplex ELISA services that enable simultaneous detection of up to 16 different analytes in a single sample.

These ELISAs provide chemiluminescent results on each analyte and allow for a global understanding of ongoing immune responses in diseases ranging from cancer to autoimmunity. Evaluation of one given inflammatory molecule in the context of several others, repeated measurements of the same cytokine panels in the same subjects under different treatment assay conditions, and reliable detection of different proteins across a broad range of concentrations are just some of the benefits provided by this multiplex service.

High Sensitivity & Specificity using low sample volumes.
Cost-Effective Quantification of Multiple Analytes.

- Variety of chemokine and cytokine panels already available for human and mouse targets
- Unique and customizable array of analytes including Type I, II, & III IFNs, and other key cytokines crucial to innate and adaptive immunity
  - Compatible with autoimmune sera, plasma, tissue culture media (TCM), and buffer samples
  - Provides analyte sensitivity to as low as 5 pg/ml
  - Reproducible results with <15% intra- and inter-assay CVs for most analytes in difficult matrices, including autoimmune serum and plasma
VeriPlex Human Cytokine 16-Plex ELISA Panel

- Simultaneously detect and measure:
  - Type I, II, & III IFNs: IFN-α, IFN-β, IFN-γ, IFN-λ1/2/3
  - Key cytokines: TNF-α, IL-1α, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17, IL-23
- Compatible with serum and plasma from autoimmune disease patients
- Detects multiple subtypes of Human IFN-α at different sensitivities
- Detects all 3 major subtypes of IFN-λ

This product has been developed to perform well using samples that are known to exhibit false positives in immunoassays. Such samples may contain human anti-mouse antibodies, anti-nuclease antibodies, and/or rheumatoid factor. The use of this product has been evaluated using serum samples from Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) patients.

VeriPlex Human Interferon 9-Plex ELISA Panel

- Quantify 9 different analytes at once:
  - Type I, II, & III IFNs: IFN-α, IFN-β, IFN-γ, IFN-λ1/2/3, IFN-ω
  - Related cytokines: TNF-α, IL-1α, IL-6, IP-10
- Compatible with serum, plasma, tissue culture media (TCM), and buffers
- Sample volume of only 50 μl required to quantify all 9 analytes in a single assay

This product may be used with samples that are known to exhibit false positives in immunoassays. Such samples may contain human anti-mouse antibodies and rheumatoid factor. The use of this product has been evaluated using serum samples from Rheumatoid Arthritis (RA) patients.
Cell-Based Assay Services

Cell-based assay tools are key to understanding cellular mechanisms in a biologically relevant context. PBL offers a targeted portfolio of cell-based assay services to provide you with the most relevant information and facilitate advancement of your research.

Whatever your cell-based study needs, PBL will work with you to determine the most suitable assay program to meet your goals. PBL’s services team is a trusted partner of midsize and major biotech and pharma companies worldwide.

Selected capabilities:

- Cytokine/Chemokine Bioactivity Assays
  - Cell Proliferation & Anti-Proliferation
  - Cytokine Secretion
  - Chemotaxis and Enzyme Induction
- Antiviral Assays (AVA)
  - Cytopathic Effect Inhibition (CPE)
  - Neutralizing Antibody Assay
  - Reporter Gene Constructs and Systems
Cytokine/Chemokine Bioactivity Assays

**Proliferation & Anti-Proliferation Assays**

Growth promotion and inhibition assays are often used to measure cytokine and drug activity profiles. Such Proliferation and Anti-Proliferation (AP) assays utilize a variety of cell lines and protein or small molecule stimuli to address specific applications and clinical indications. EC\(_{50}\) or IC\(_{50}\) results are provided.

The top graph to the right shows OVCAR-3 human adenocarcinoma cell line grown in the presence of IFN-alpha. Cellular antiproliferation was quantified using soluble tetrazolium reagent. The bottom graph to the right shows TF-1 human erythro-leukemia cell line grown in the presence of GM-CSF. Cellular proliferation was quantified using soluble tetrazolium reagent. For both graphs, the quantity of reduced reagent product is directly proportional to viable cell number. EC\(_{50}\) was determined by nonlinear regression using a four-parameter (variable slope) curve fit.

**Cytokine Secretion Assays**

Accurate measurement of the ability of a sample to induce cytokine production and/or release from cell lines or isolated donor cells is the standard assay for certain applications, such as measurement of IL-18 activity. The graph to the right shows a natural killer cell model, NK-92, stimulated to produce IFN-Gamma by incubation with Type I IFNs. The data is graphed as a function of IFN-Alpha concentration in the bioassay. EC\(_{50}\) was determined by nonlinear regression using a four-parameter curve fit.
Antiviral Assays

Cytopathic Effect Inhibition (CPE) Assays

- Frequently used systems:
  - Human A549 / EMCV
  - Bovine MDBK
  - Mouse L929 / VSV
- High level of assay sensitivity and accuracy

The standard bioassay used to determine the biological activity of interferon (IFN) encompasses measurement of the protection of cells from the cytopathic effect (CPE) of certain viruses. CPE assay services may be used to measure the antiviral activity of IFN-α, β, ω, γ, and λ in human samples, as well as in a variety of other species, and in multiple matrices.

CPE assays are inherently complex due to the metabolic state of cells, virus replication, and the ability of IFN to protect cells. Our years of knowledge and expertise in CPE assays will reduce variability and provide you with reproducible quality results.

Results are provided either as a graphical representation of dye binding which allows slope and parallel line analysis, or visually determined from microscopic examination of the CPE and determination of the dilution of test samples which protects 50% of the cells.

Neutralizing Antibody Assays

- Suitable for serum, plasma, cell culture media, or tissue culture media samples

The standard method of accurately determining whether a sample may inhibit the biological activity of cytokines is the neutralization assay. Results, provided either as a visual read or in a graphical format, reveal the ability of a sample to block the activity of IFN added at a specific concentration which provides 100% cell protection.

May be used as an anti-drug antibody (ADA) assay for certain therapeutics.
iLite® Assay-Ready Cell Line Assay Service

Reporter Gene Constructs and Systems

- Novel and highly specific cell lines deliver unrivaled specificity as well as precision and sensitivity
- Dual reporter gene technology allows for normalization of serum matrix effects as well as cell counts
- Increased applicability of cell-based assays

Assay-ready cell lines are genetically engineered with target specific reporter gene constructs that respond specifically to the analyte of interest with a luminescent readout. Only the target analyte can trigger a signal through its specific cell membrane receptor, thus generating an analyte specific signal.

Bioactivity is calculated as the difference between the specific signaling generated by the target receptor (Firefly Luciferase) and that generated by a secondary reporter gene’s signal (Renilla Luciferase). The secondary luciferase readout enables normalization of each individual readout according to cell number and thereby accounts for any potential matrix effects. These factors collude to create a sensitive, specific, and precise assay.

Assay-ready cells do not require culturing or continuous cell maintenance and can be used immediately, eliminating many of the limitations of “conventional” cell-based assays.
PBL’s reagent development services offer customized production of cytokines, interferons, antibodies, and other proteins.

**Selected capabilities:**
- Recombinant Protein Production and Purification (bacterial & mammalian)
- Cytokine & Antibody Production and Characterization
- Hybridoma, Polyclonal Antibody, and Cell Line Development
- Protein & Antibody Labeling and Biophysical Characterization

With a wealth of knowledge on immunological interactions, we offer protein biophysical characterization services which can determine the kinetic characterization of protein-protein interactions. These services include determination of:

- Affinity of protein-protein interactions
- Association and dissociation rates of protein-protein interactions
- Antibody-antigen specificity and affinity

PBL offers biophysical characterization of antibody affinity for a ligand or the measurement of antigen:antibody $K_{d}/K_{off}$ rates. To accelerate assay development, we also determine which antibodies bind to antigens in a sandwich format. Binning—determination of antibodies which bind to non-overlapping epitopes—is performed using multiple hybridoma clones.

The graph to the right shows the association and dissociation of IFN to a surface-immobilized antibody using varying concentrations of IFN. The raw data is shown in blue and the model response in red.
Custom Assay Design & Development

PBL assay development services can be tailored to your specific needs. Assays are developed for research use or to a standard which supports subsequent GLP validation.

You can be confident that PBL will provide a viable solution that will maximize the data from your samples during a development project. We will work alongside you to define essential steps, reagents required, and identify potential issues before they occur. From proof of principle to technology transfer, our experience will ensure we deliver a robust and sensitive assay solution for your specific application needs.

Cell-based Assay Development

Cell-based assay development services offered, but not limited to, include:

- Custom cytopathic effect, anti-proliferative, and growth promotion assays
- Development of reporter gene systems or identification of cell lines to monitor activity of the compound of interest
- Neutralization of bioactivity by antibodies which is relevant to identification of bioactive monoclonal antibodies (MAbs) and monitoring of immunogenicity

Immunoassay Development

Custom immunoassays and ELISAs to support:

- Pharmacokinetic and pharmacodynamic studies
- Toxicity and immunogenicity studies
- Direct, sandwich, or competitive ELISA assay development

PBL Assay Science has extensive experience and depth of knowledge in cytokine and interferon related research as well as immunological assay development. The unique perspective of our scientists ensures we deliver reliable results throughout projects. With our attention to detail and collaborative approach, we provide uncompromising quality from a company you can trust.

To explore how PBL may help or to discuss an upcoming project, please contact us.