RESEARCH. DELIVERED.

PBL ASSAY SERVICES

pbl ASSAY SCIENCE

ble Results Reliable Results
s Deeper Insights Deeper I
Decisions Better Decisions

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
  - Quanterix Simoa®
  - EMD Millipore (Singulex) Erenna®
- Singleplex & Multiplex Immunoassays
  - Meso Scale Discovery® ECL

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

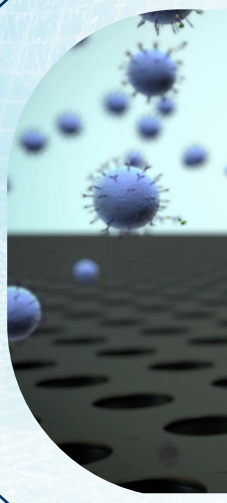
As SMEs in immunoassays, cytokines, and interferons, we can help turn your data into actionable knowledge.

Entrusting your sample testing analysis to PBL's experienced team of dedicated assay services scientists and quality control experts ensures trustworthy and accurate results.

Our scientists communicate directly with you building a transparent, collaborative environment and eliminating costly missteps.

Inquire with our Assay Services team to learn more about our specific capabilities and how we can help advance your research.

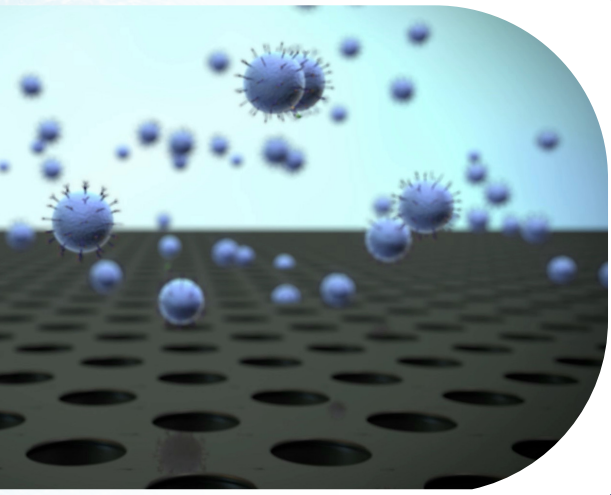
P +1 732-777-9123
F +1 732-777-9141
E assayservices@pblassaysci.com
W pblassaysci.com



Quantitative Immunoassays

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.

For many of your sample testing and screening needs, PBL can help you measure your analyte(s) of interest. From state-of-the-art technologies quantifying proteins 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 Cytokine & Biomarker Quantification
  - Quanterix Simoa®
  - EMD Millipore (Singulex) Erenna®
- Single and Multiplex Immunoassays
  - Meso Scale Discovery® Electrochemiluminescence (MSD-ECL)
  - Single Analyte ELISAs
  - Multiplex Screening ELISAs

Quanterix Simoa®

Ultra-Sensitive fg/ml Biomarker Quantification Services

High Precision. Unprecedented Sensitivity. Digital Readout.

Take your detection to the next level.

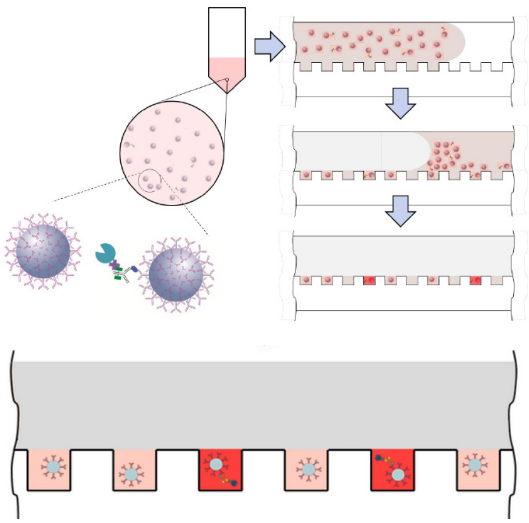
- Single Molecule Array technology delivers **femtogram per milliliter** (fg/ml) level sensitivity for measurement of low-abundance biomarkers in **serum**, **plasma**, and other matrices
- Unique technology provides robust detection for single analytes and select **multiplex** panel options
- High precision service offers accurate and reproducible data with < 10% inter- and intra-assay CVs
- Over 50 analytes available including IL-17A, TNF-α, IL-23, IP-10, TRAIL, IL-4, IL-6, IL-8, IL-10, IL-12p70, Aβ40, Aβ42, Tau, and NF-LIGHT®

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

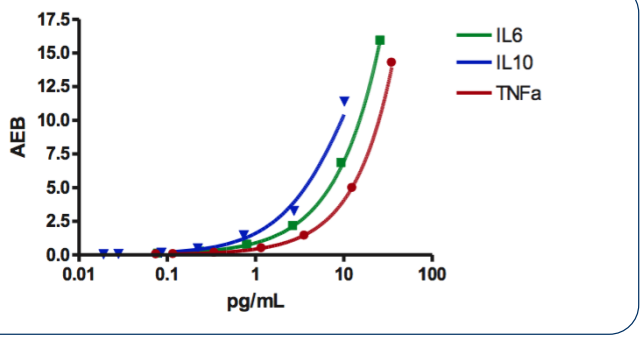
The combination of this instrument's markedly heightened sensitivity and its capacity for digital analyte measurement ensures accuracy and precision across a wide dynamic range and minimizes sample volume requirements.

†Powered by Simoa® Technology, Quanterix Corporation

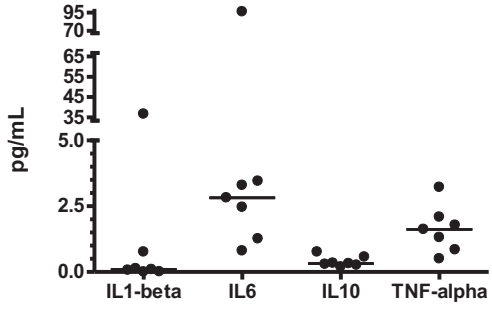
Technology Overview



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

EMD Millipore (Singulex) Erenna®

Ultra-Sensitive fg/ml Cytokine Testing Services

Accurate and Robust Measurement. Increase your Understanding.

Detect at unprecedented levels.

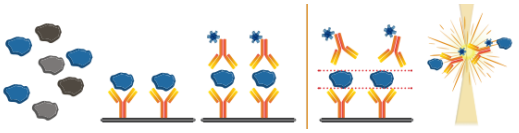
- Cytokine analysis utilizing Single Molecule Counting (SMC) technology enables **sub-pg/ml** sensitivity for accurate measurement of low abundance biomarkers in normal or disease **serum/plasma**
- Minimal sample dilution requirement with 4-Log+ dynamic range
- High precision cytokine immunoassays provide reproducible data
- Extensive analyte menu of over 40 different cytokine assays covering several disease areas including IFN-γ, IL-1a, IL-1b, IL-4, IL-6, IL-8, IL-10, IL-13, IL-15, IL-17A, IL-17F, IL-17 heterodimer, IL-23, GLP-1, and TNF-α

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.

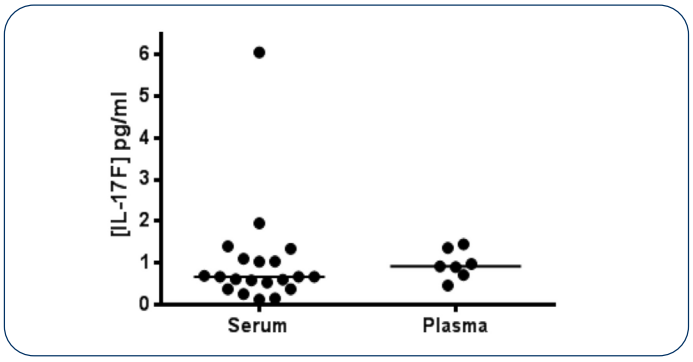
An increased understanding of the role and regulation of cytokines in disease states results from sensitive profiling and characterization of their activities in biological responses. Singulex technology can be instrumental in furthering our collective understanding of low-abundance cytokines in complex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

††Powered by the Erenna® Immunoassay System, EMD Millipore Inc.

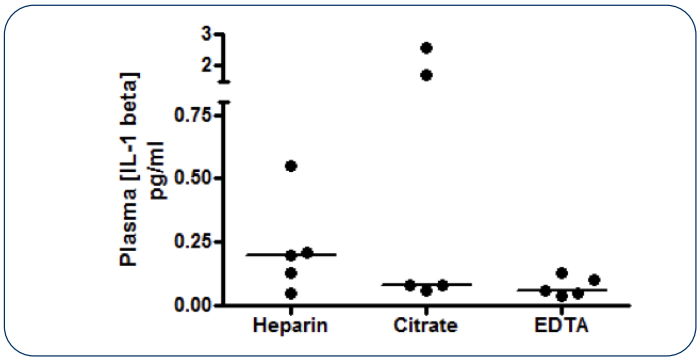
Technology Overview



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 antibodes, 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.



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

Meso Scale Discovery® Electrochemiluminescence (MSD-ECL)

High Performance Protein Detection Services in Single and Multiplex Formats

Broad dynamic range. Minimal background. High sensitivity.

Enhanced signal capture achieved through luminescent detection.

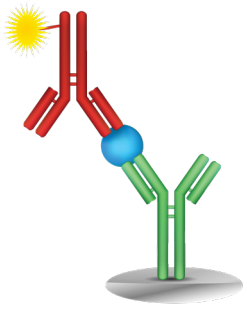
- Ability to target low analyte levels in **small sample volumes**
- Robust **matrix tolerance** including serum, plasma, and TCM, as well as various complex biological matrices
- Measure high and low abundance analytes (normal and elevated levels) within the same sample with **5-Log+ dynamic range**
- Over 180 **validated** fit-for-purpose singleplex and preconfigured multiplex panel ELISAs available with excellent performance specifications and lot-to-lot consistency

PBL offers high precision protein quantification services for biomarker detection in a broad range of sample matrices for Human, Non-Human Primate (NHP), Mouse, Rat, and Canine samples. PBL utilizes MSD's validated assay kits (V-Plex) to quantify up to 54 analytes within a single sample. This enables simultaneous comparison of the expression of pertinent cytokines, chemokines, and other biomarkers in samples from diseased and normal patients at different intervals.

Multiplex immunoassays on the MSD platform††† maintain the sensitivity and performance offered by singleplex ELISAs while providing additional benefits such as cost-savings and targeting of several related analytes in a single sample. The ability to quantify compatible analytes while requiring no more than 25 µl of neat sample ensures efficent use of precious sample.

†††Powered by the Meso Scale Discovery® technological platform.

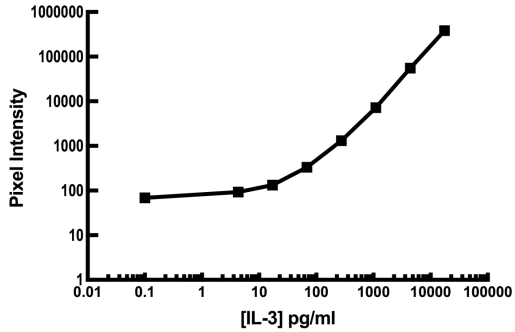
Technology Overview



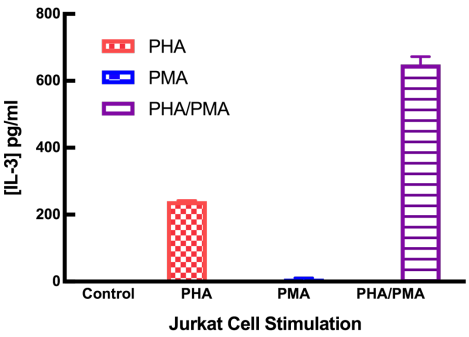
Unique detection technology utilizes SULFO-TAG™ labels which emit light upon electrochemical stimulation. The intensity of light generated is captured via CCD camera and pixel intensities quantified. This system provides the basis for achieving measurement of a several log range of biomarker expression levels in a variety of matrices.

Broad Range of Applications

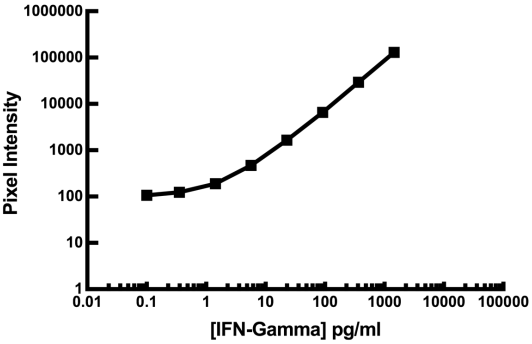
- Cytokines & Chemokines
- Cardiac Biomarkers
- Immunogenicity
- Immunology
- Inflammation
- Intracellular signaling
- Metabolic
- Neurodegeneration
- Oncology and Cancer
- Toxicity
- ...and more



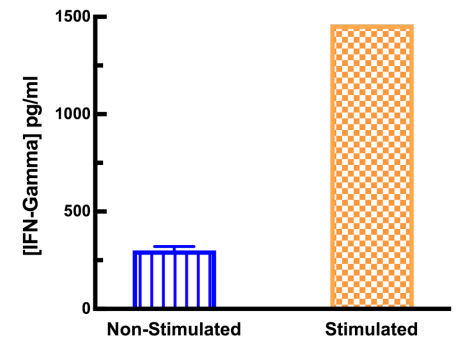
Human IL-3 standard curve demonstrates the broad dynamic range characteristic of MSD immunoassays.



IL-3 production by Jurkat cell line after stimulation by phytohemagglutinin (PHA), phorbol myristate acetate (PMA), and both agents.



Human IFN-Gamma standard curve exhibits a wide dynamic range allowing for sensitive measurement in undiluted samples.



Human IFN-Gamma released from NK-92 cells upon exposure to IL-2.

Single Analyte ELISA Service

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

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

PBL ELISAs:

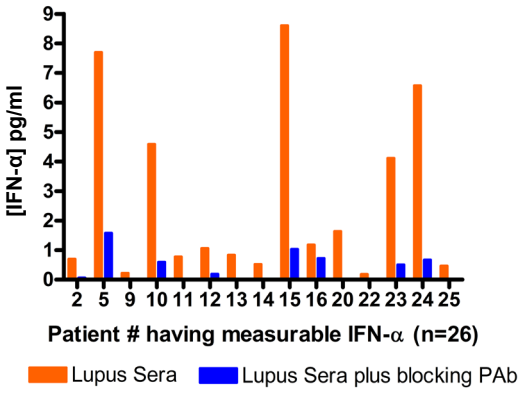
- High sensitivity ELISAs available for a comprehensive range of species and analytes
- Compatible with a variety of matrices including serum, plasma, tissue culture media (TCM), and cell lysates
- Matrix tolerance for autoimmune disease sera*
- Reproducible results with low intra- and inter-assay CVs

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:

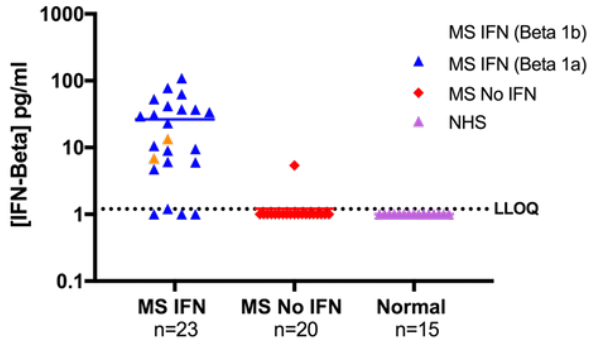
- **Human** IFN-Alpha*, Beta*, Gamma, Lambda 1/2/3, Omega, Gamma Receptor 1
- **Mouse** IFN-Alpha, Beta, Lambda 2/3
- **Cynomolgus/Rhesus** IFN-Alpha, Beta

High Sensitivity Human IFN Alpha All Subtype ELISA



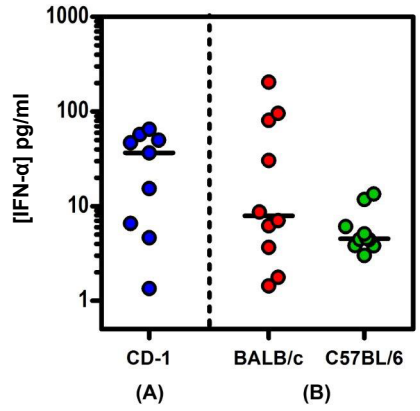
IFN-Alpha specific signal was measured in sera of Lupus patients using the 41115 ELISA. Blue bars represent non-specific signal, i.e. signal not blocked by Anti-IFN-Alpha PAb.

High Sensitivity Human IFN Beta Serum ELISA



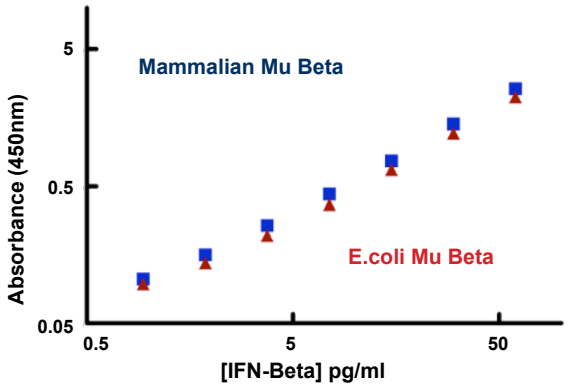
Levels of IFN-Beta Quantified in Normal Human Sera (NHS) and Multiple Sclerosis (MS) Patient Sera using the 41415 ELISA. IFN-Beta was quantifiable in 87% of MS patients on IFN-Beta therapy and 5% of patients on other therapies. All samples found to be below LLOQ are plotted at the level of 1.0 pg/ml.

High Sensitivity Mouse IFN Alpha ELISA



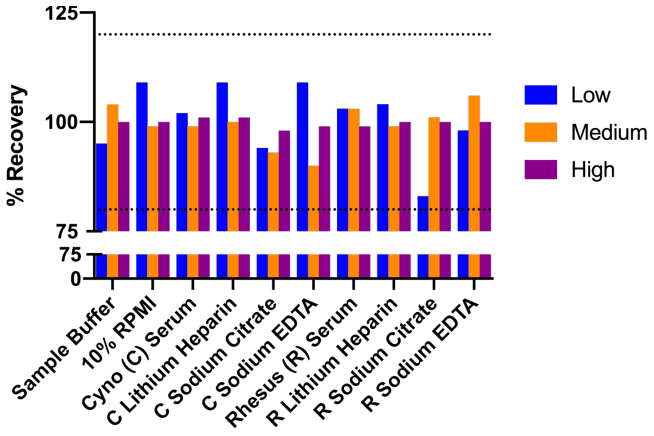
Levels of endogenous mouse IFN-α quantified in pooled plasma (A) and individual sera (B) using the 42115 ELISA.

High Sensitivity Mouse IFN Beta Serum ELISA



Comparison of ELISA reactivity of Mammalian- and E.coli-expressed mouse IFN-Beta using the 42410 ELISA.

Cynomolgus/Rhesus IFN Alpha ELISA



Three concentrations of Cynomolgus/Rhesus IFN-Alpha were added to serum, plasma (Lithium Heparin, Sodium Citrate, Sodium EDTA), TCM containing 10% fetal bovine serum, and kit sample buffer. Percent recovery was determined using the 46100 ELISA. IFN-Alpha was measureable with an accuracy of +/- 20% of the expected value.

Cynomolgus IFN Beta ELISA

	Low	Medium	High
Intra-Assay CV	2.5%	1.6%	2.8%
Inter-Assay CV *Operator 1*	5.4%	4.3%	2.3%
Inter-Assay CV *Operator 2*	4.4%	3.4%	5.8%
Average % Recovery	93.7%	97.1%	92.4%

Cynomolgus IFN Beta was spiked into a single lot of normal cynomolgus serum at three different concentrations and analyzed using the 46415 ELISA.

Multiplex ELISA Screening Service

High Sensitivity & Specificity using low sample volumes.

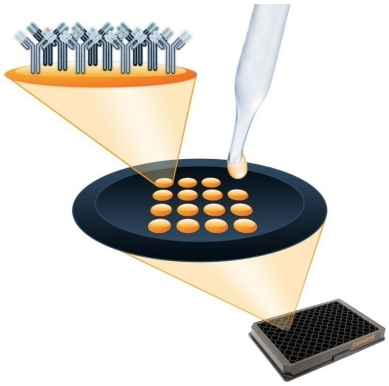
Cost-Effective Quantification of Multiple Analytes.

- Variety of chemokine and cytokine panels available for human and mouse targets
- Unique array of analytes including Type I, II & III IFNs, and other key cytokines crucial to innate and adaptive immunity
- Compatible with **autoimmune sera**, plasma, and tissue culture media (TCM)
- Sample volumes of only 50 µl required to quantify all analytes in a single assay
- Reproducible results with < 15% intra- & inter-assay CVs for most analytes in difficult matrices

PBL's cost-effective multiplex ELISA services enable simultaneous detection of up to 9 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.

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.

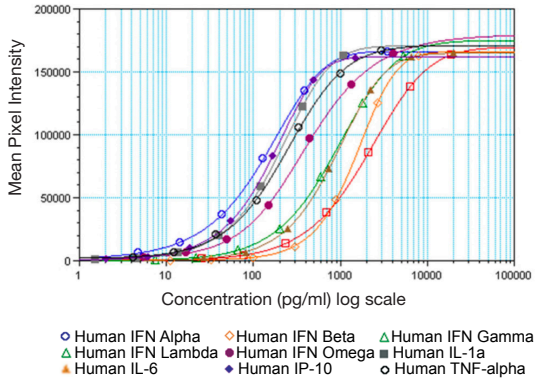
Category	Multiplex Technology	Electro-Chemiluminescent	Bead-Based
Data Output	864 data points / test	864 data points / test	1000 data points / test
Sample Size	15 - 50 µl / test	50 - 100 µl / test	12.5 - 50 µl / test
Sample Type	Many Types	Many Types	Difficulty with serum and other viscous samples
Variation	Low Variability ≤ 7% CV	Acceptable Variability ≤ 10% CV	Bead Lot-to-Lot Variability
Sensitivity	Low pg/ml	Low pg/ml	Low pg/ml

VeriPlex Human Multiplex ELISA Panel (9-Plex)

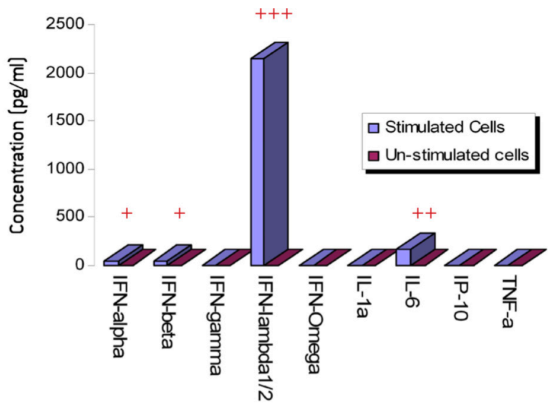
This kit has been developed to perform well using samples that are known to exhibit false positives in immunoassays. Such samples may contain human anti-mouse antibodies, anti-nuclease antibodies, and/or rheumatoid factor. The 9-Plex has been evaluated using serum samples from Rheumatoid Arthritis (RA) patients.

VeriPlex Human Interferon 9-Plex ELISA Analytes				
IFN-α	IFN-β	IFN-γ	IFN-λ1/2/3	IFN-ω
TNF-α	IL-1α	IL-6	IP-10	

Representative Analyte Standard Curves on Human Cytokine 9-Plex ELISA



Representative *in vitro* Experimental Data



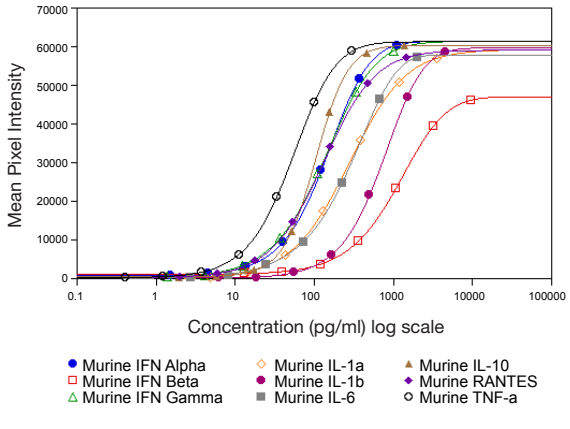
Cytokines released by A549 human lung epithelial cells following infection with EMCV at MOI 0.001 over 48 hours.

VeriPlex Mouse Multiplex ELISA Panel (9-Plex)

This kit has been developed to simultaneously detect key cytokines released upstream and downstream of interferon signaling in multiple matrices including tissue culture media (TCM), mouse serum, and mouse plasma.

VeriPlex Mouse Cytokine 9-Plex ELISA Analytes		
IFN-α	IFN-β	IFN-γ
IL-1α	IL-1β	IL-6
IL-10	RANTES	TNF-α

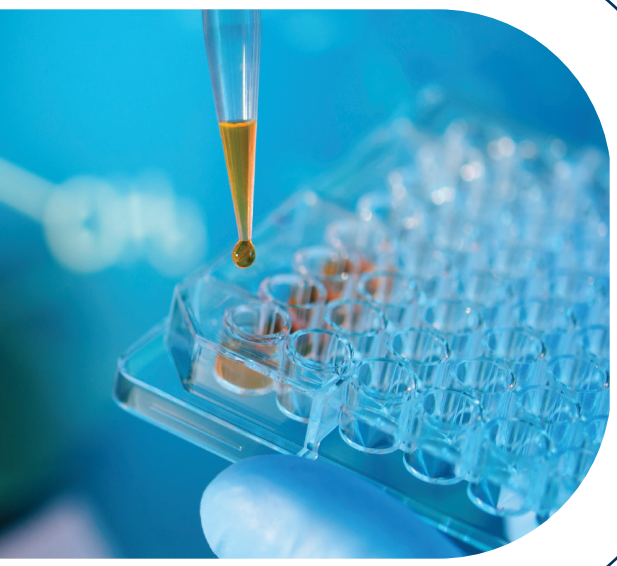
Representative Analyte Standard Curves on Mouse Cytokine 9-Plex ELISA



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:

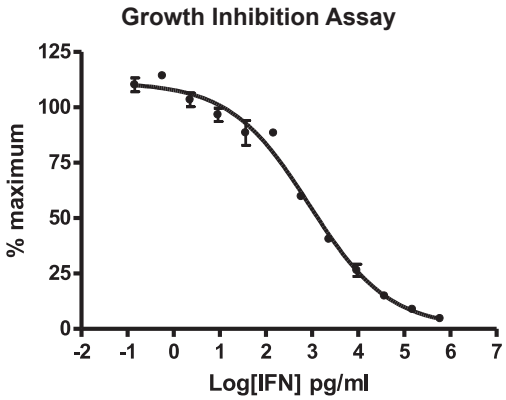
- Cytokine/Chemokine Bioactivity Assays
  - Cell Proliferation & Anti-Proliferation
  - Cytokine Secretion
  - Chemotaxis and Enzyme Induction
- Antiviral Assays (AVA)
  - Cytopathic Effect Inhibition (CPE)
  - Neutralizing Antibody Assay
  - Reporter Gene Constructs and Systems

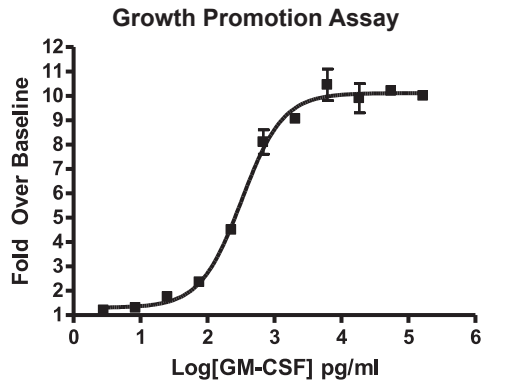
Cytokine/Chemokine Bioactivity Assays

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.

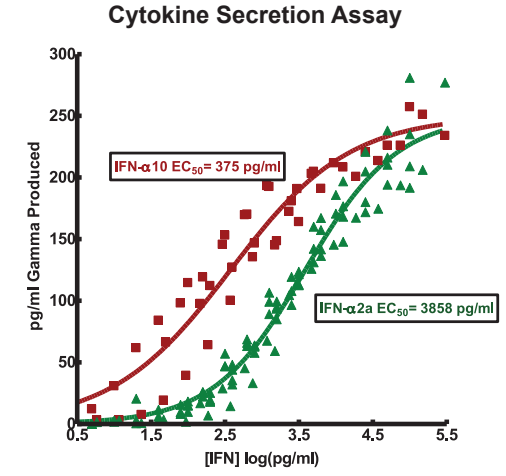
The top graph to the right shows OVCAR-3 human adeno-carcinoma cell line grown in the presence of IFN-alpha. Cellular antiproliferation was quantified using soluble tetrazolium reagent. The bottom graph to the right shows TF-1 human erythro-leukemia cell line grown in the presence of GM-CSF. Cellular 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

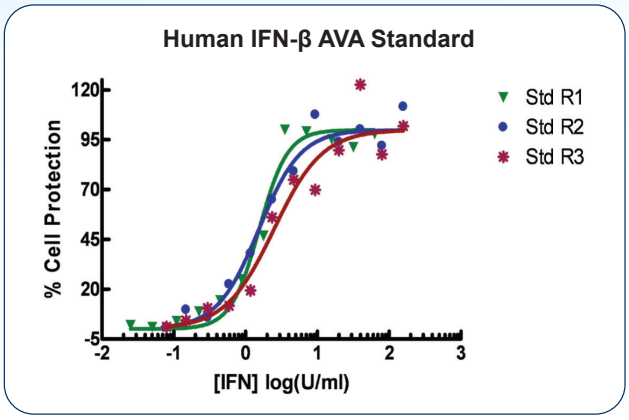
Cytopathic Effect Inhibition (CPE) Assays

- 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.



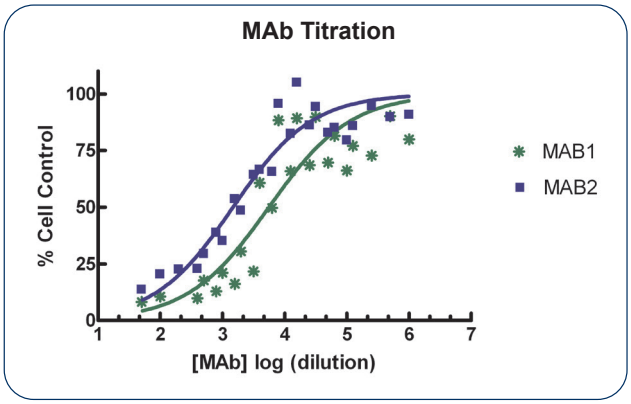
IFN-beta was titrated in a cytopathic effect inhibition (CPE) assay on A549 human lung carcinoma cells with encephalomyocarditis virus (EMCV). In this antiviral assay, approximately 1 unit/ml of interferon is the quantity necessary to produce an inhibition of the cytopathic effect of 50% (EC_{50}). Units of IFN activity were determined colorimetrically with respect to an international reference standard for human interferon.

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.

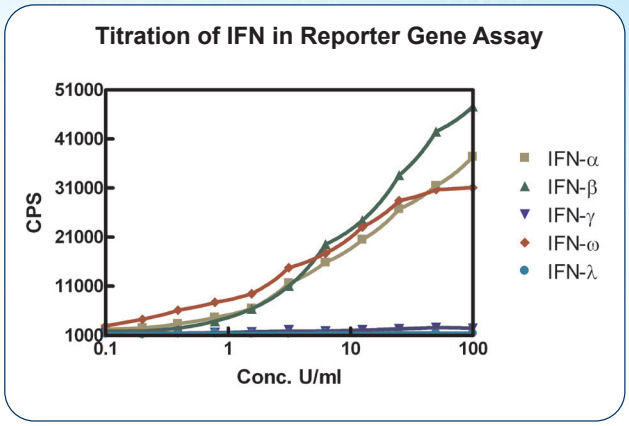


Representative neutralization curves of two different monoclonal antibodies to interferon in a cytopathic effect inhibition (CPE) assay to determine neutralization titer. One neutralization unit is the amount of antiserum or antibody (NAb) required to neutralize one unit of human interferon to a 50% endpoint in the bioassay. Neutralizing Units (NU) are determined with respect to the international reference standard.

iLite® Assay-Ready Cell Line Assay Service

Reporter Gene Constructs and Systems

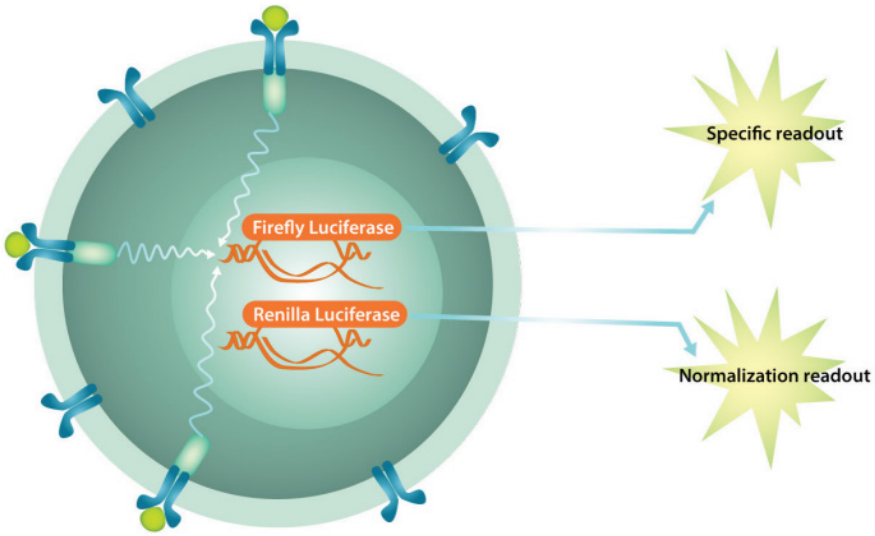
- Novel and highly specific cell lines deliver unrivaled specificity as well as precision and sensitivity
- Dual reporter gene technology allows for normalization of serum matrix effects as well as cell counts
- Increased applicability of cell-based assays



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.



Reagent Development Services

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

Protein & Antibody Development and Characterization

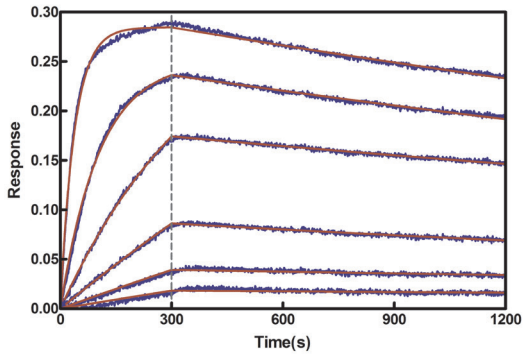
With a wealth of knowledge on immunological interactions, we offer protein biophysical characterization services which can determine the kinetic characterization of protein-protein interactions. These services include determination of:

- 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_{on}/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 as determined by real-time Biolayer Interferometry (BLI) using varying concentrations of IFN. The raw data is shown in blue and the model response in red.

IFN Binding to and Dissociation from a Surface-Immobilized Monoclonal Antibody Evaluated by BLI



Additional PBL Services

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.

PBL Assay Science
131 Ethel Road West, Suite 6, Piscataway, New Jersey 08854 USA
P +1 732-777-9123
F +1 732-777-9141
E assayservices@pblassaysci.com
W www.pblassaysci.com