

Cell analysis

# Biomarker quantitation guide

## Quantitate protein:

ELISA | Antibody pairs | ProQuantum HS immunoassays | ProcartaPlex immunoassays

## Quantitate mRNA:

QuantiGene multiplex and singleplex gene expression assays

The Invitrogen™ portfolio offers a variety of assays for singleplex and multiplex quantitation of both proteins and mRNA. Our extensive selection of Invitrogen™ assays includes:

- ELISA and antibody pair kits
- ProQuantum™ high-sensitivity immunoassay kits
- ProcartaPlex™ multiplex and simplex immunoassay kits
- QuantiGene™ Plex and Singleplex gene expression assays

Additionally, Thermo Fisher Scientific supports your quantitation assay needs with special services, accessory reagents, and instruments for a comprehensive offering. Use this guide to help identify your needs, then contact your sales representative if you have questions.



You can also visit

**[thermofisher.com/immunoassays](https://www.thermofisher.com/immunoassays)**

# Contents



Biomarker background	4
Highly referenced kits for multiple research areas	
<hr/>	
Biomarker quantitation assay platforms	8
Products tailored to meet your needs	
<hr/>	
Single-biomarker quantitation of protein targets	10
Precoated ELISA kits	
Uncoated ELISA and antibody pair kits	
ProQuantum high-sensitivity immunoassay kits	
<hr/>	
Single-biomarker quantitation of mRNA targets	17
QuantiGene Singleplex kits	
<hr/>	
Accessory reagents and equipment	18
Microplate washers	
Microplate readers	
<hr/>	
Multiplex biomarker quantitation: product overview	20
Assays and instruments for protein and RNA targets	
<hr/>	
Multiplex biomarker quantitation of protein targets	21
Luminex xMAP technology	
ProcartaPlex multiplex immunoassay kits	
ProcartaPlex high-sensitivity immunoassay kits	
ProcartaPlex mix and match panels	
Sample measurement service	
<hr/>	
Multiplex biomarker quantitation of mRNA targets	32
QuantiGene Plex multiplex assays	
<hr/>	
Luminex instrumentation	37
<hr/>	
Additional solutions for gene expression analysis	38
<hr/>	
High-throughput quantitation	40
ProcartaPlex and QuantiGene 384-well assays	
Laboratory automation solutions	
<hr/>	
Immunoassay special services	45
<hr/>	

# Biomarker background

## Highly referenced kits for multiple research areas

To facilitate the investigation of a wide variety of biological markers, the Invitrogen portfolio has a broad menu of immunoassays to easily detect and quantify proteins and RNAs. This guide describes all the options available for single- and multi-biomarker quantitation assays, as well as the appropriate instrument platforms for each type of assay, so you can choose the platform you need to publish with confidence. Products are available for important research areas, including inflammation, cancer, neurobiology, immunoglobulin isotyping, SARS-CoV-2 serology, cardiovascular diseases, metabolism, and more.

### Inflammation

As part of the immune response, inflammation plays an important role in defense against pathogens, such as viruses, bacteria, fungi, and other parasites. It involves the coordinated communication of different immune cells and blood vessels through an intricate cascade of cellular and molecular signals (Figure 1). Acute inflammation is a short-lived response that is characterized by extravasation of leukocytes, erythrocytes, and plasma components into the injured tissue. Figure 1 shows a simplified representation of cytokine interactions during inflammation.

If left unchecked, the acute inflammatory process can lead to chronic inflammation, which has been linked to obesity, type 2 diabetes, allergy, atherosclerosis, arthritis, Alzheimer's disease, autoimmune diseases, and cancer [1,2,3]. While the process of acute inflammation is well defined, the causes of chronic inflammation and associated molecular and cellular pathways are not well understood [4]. Access to qualified and reliable immunoassays to aid in biomarker and disease research can help lead the way to a better understanding of such pathways, and ultimately, better treatments for devastating diseases.

#### Inflammation research

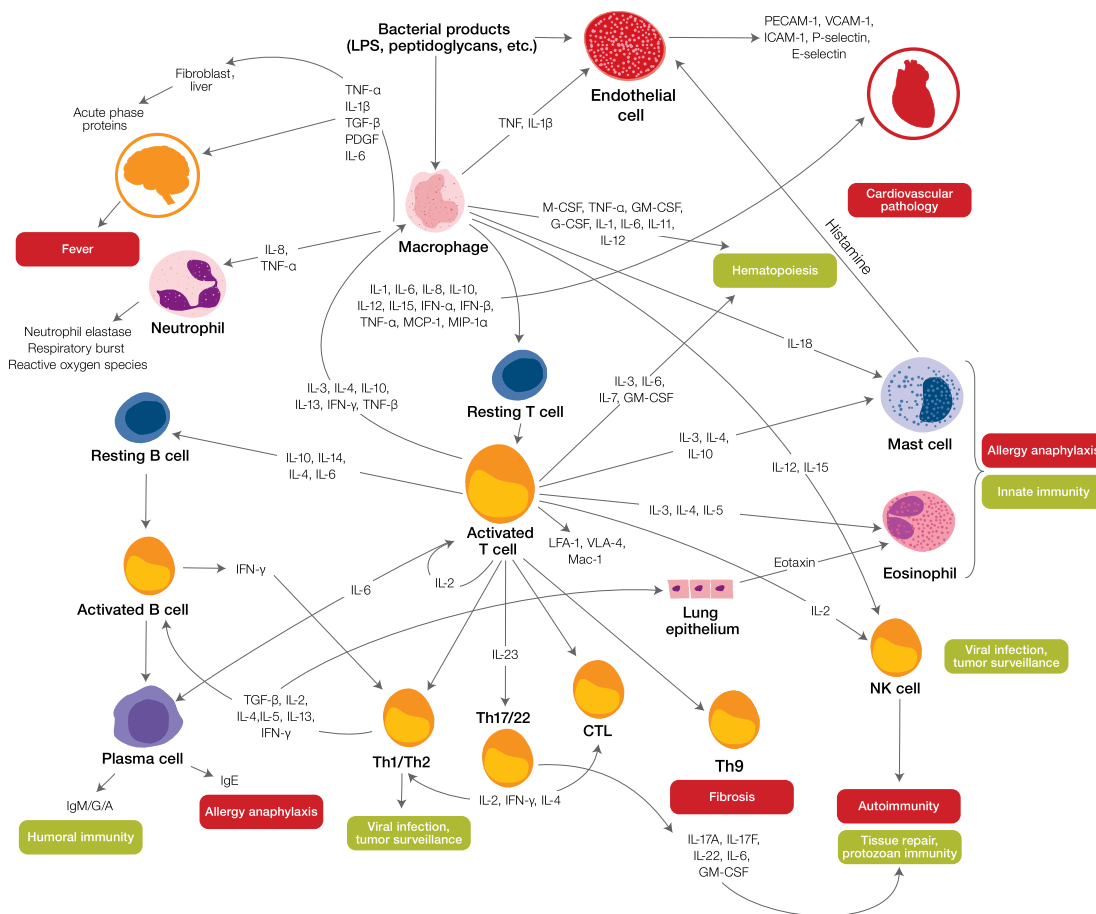


Figure 1. Interaction of cytokines during inflammation.

## Hallmarks of cancer

Cancer is a leading cause of death worldwide and has become a major public health issue in developed countries. Cancer development is a multistep process, during which the cells accumulate genetic abnormalities, especially in oncogenes and tumor suppressor genes, contributing to uncontrolled proliferation.

Hanahan and Weinberg described key changes and characteristics that occur during the transformation from a normal cell to a tumor cell; these features may be considered

hallmarks of cancer [5] (Figure 2). In addition, certain inflammatory responses have the inadvertent effect of supporting tumor functions, such as supplying the tumor microenvironments with growth and survival factors, and various enzymes that facilitate angiogenesis and aid in invasion and metastasis [5,6].



Visit our [immuno-oncology immunoassay page](#) to learn more about proteomic and genomic kits aimed specifically at supporting cancer and immuno-oncology research.

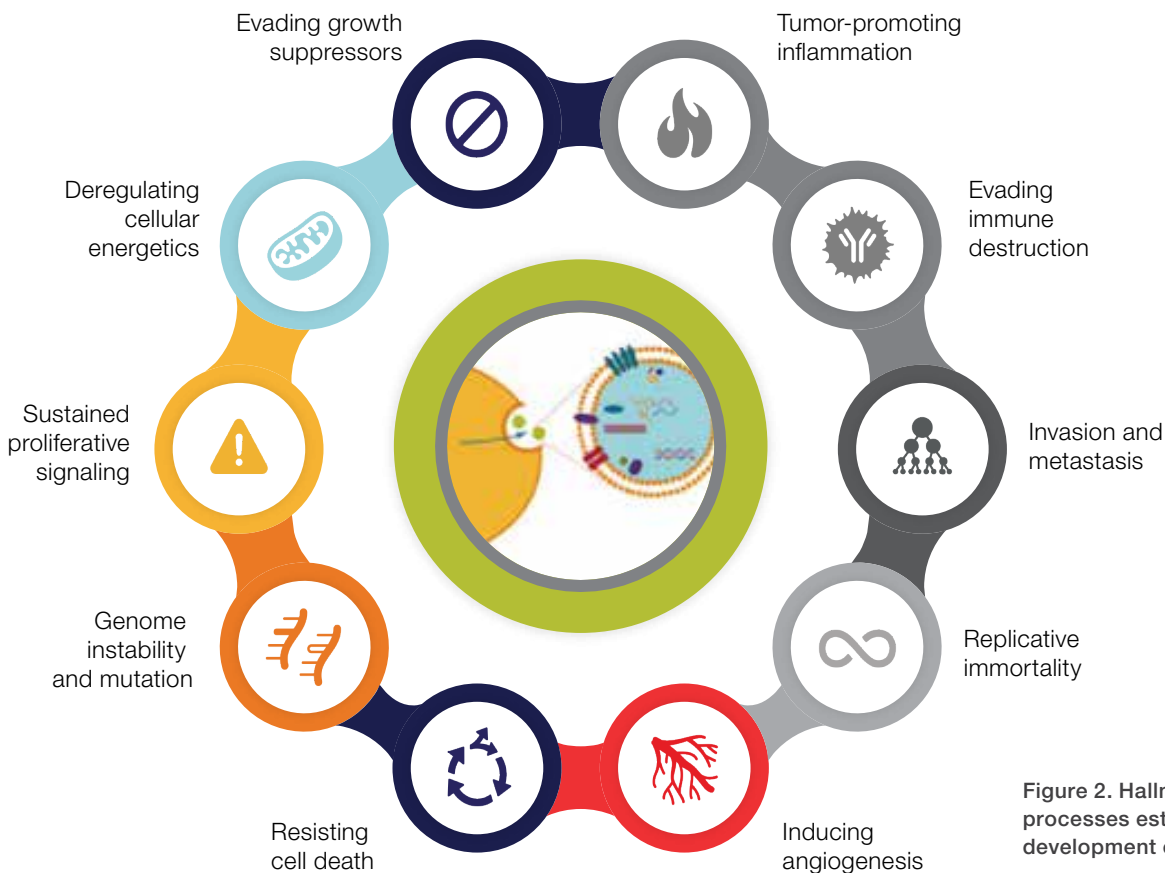


Figure 2. Hallmarks of cancer—biological processes established during the development of human tumors.

# Neurobiology

Neuroinflammation is associated with neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, which are thought to be caused by altered proteins that undergo an unfolding process followed by formation of protein aggregates that cause neuronal toxicity. One Alzheimer's disease model theorizes that initiation of disease involves damage signals that promote cellular and molecular activation and release of proinflammatory cytokines, which leads to an overactivation of protein kinases that begin the protein unfolding process [7]. Further characterization and understanding of these cellular, molecular, and protein interactions will aid in development of treatments for and prevention of neurodegenerative diseases (Figure 3).



Visit our [neurobiology immunoassay page](#) to learn more about proteomic and genomic kits supporting neurodegeneration, traumatic brain injury, and neuroinflammation research.

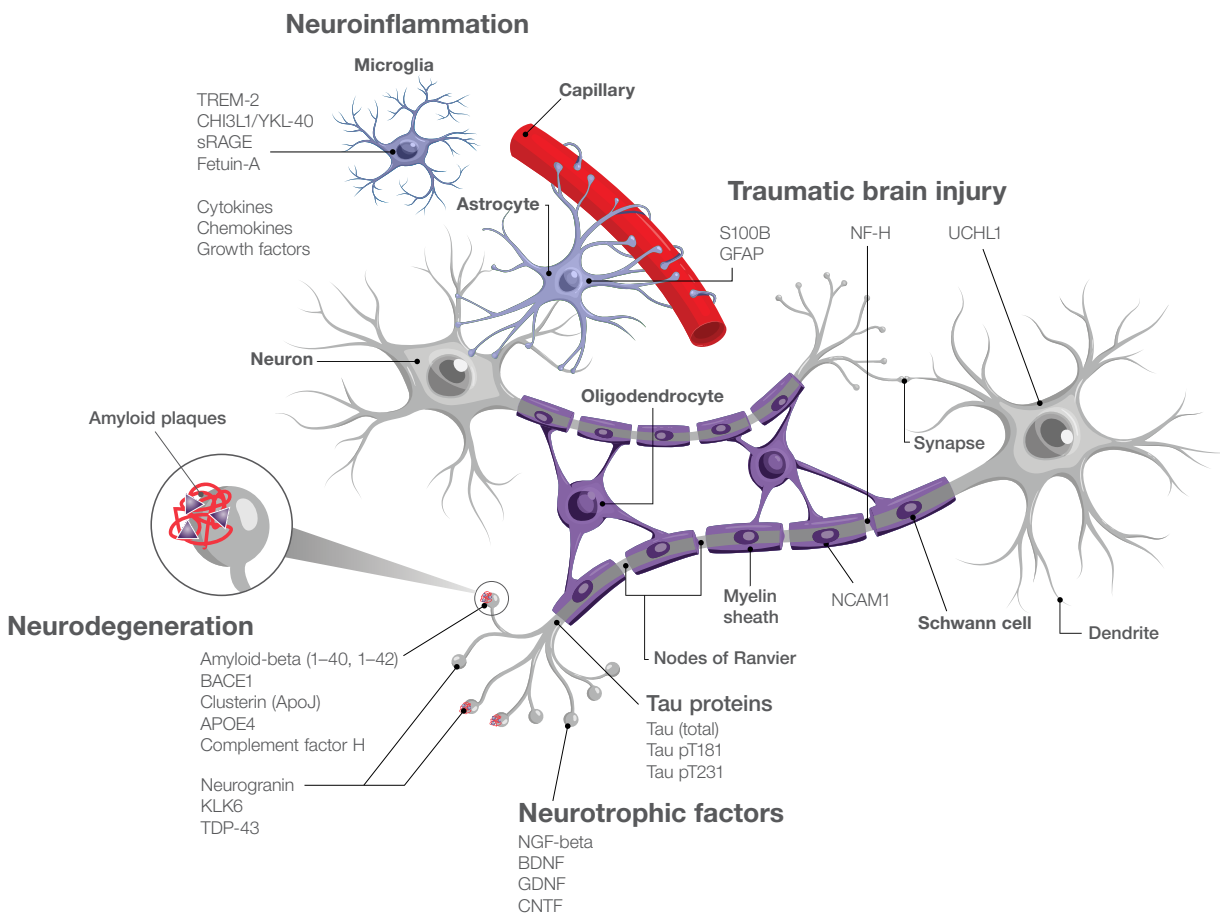


Figure 3. Major neuronal and non-neuronal cell types of the CNS and candidate biomarkers for neuroinflammation, brain injury, and neurodegeneration.

## Immunoglobulin isotyping

The various antibodies produced by plasma cells are classified by their isotypes that differ in function and antigen responses. Five major classes, or antibody isotypes, have been identified in mammals, which are categorized according to differences in their amino acid sequence in the constant region (Fc) of the antibody heavy chains (Figure 4). Antibody isotypes are further grouped into subclasses based on additional small differences in their amino acid heavy chain sequences. Determination of individual subclasses is relevant in assessing primary immunodeficiencies or immune responses, especially if the total IgG or IgA concentration is not altered.



Visit our [isotyping immunoglobulin immunoassays page](#) to learn more.

## SARS-CoV-2 serology

Serology assays enable researchers to determine if subjects have already been infected with severe acute respiratory coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19, by measuring the presence of antibodies against SARS-CoV-2 in blood (serum or plasma). A detectable level of anti-SARS-CoV-2 antibodies is dependent on the immune response and can be influenced by a variety of factors including dose of virus exposure, assessment stage post-symptom onset, age, gender, and health status. Therefore, specific and sensitive serological assays are important for accurate and reliable detection of anti-SARS-CoV-2 antibodies.



Visit our [SARS-CoV-2 serology assays page](#) to learn more.

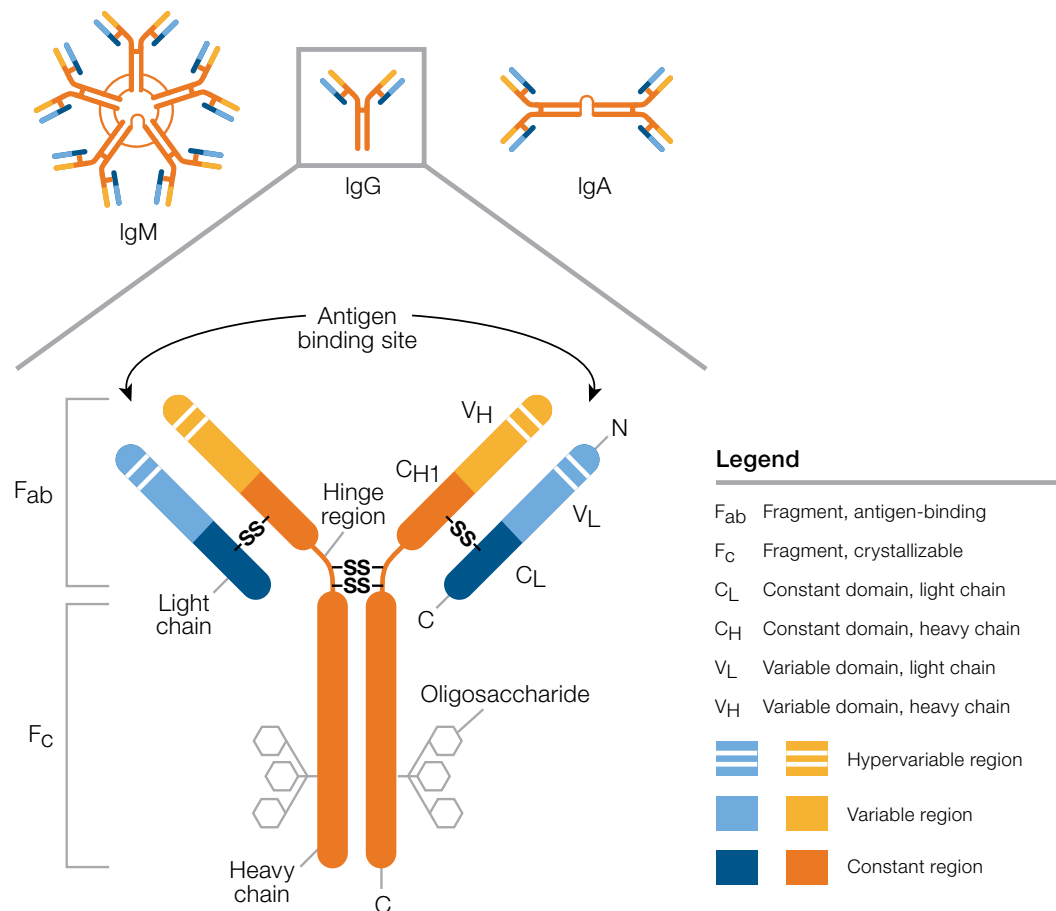


Figure 4. Annotated diagram of immunoglobulin structure.

# Biomarker quantitation assay platforms

## Products tailored to meet your needs

A variety of assay platforms are available including plate- and bead-based solutions across many different species. ELISA kit formats include complete, ready-to-use kits as well as preoptimized reagents to make your own. In addition, singleplex and multiplex options are available for protein quantitation with ProcartaPlex assays and mRNA quantitation with QuantiGene assays. See Table 1 for a component list and Table 2 for a performance comparison of our immunoassay platforms.

- **Invitrogen™ coated ELISA kits**—quantitate with confidence. Rigorously verified ELISA kits with precoated plates enable lower inter- and intra-assay variability with ready-to-use reagents that help ensure consistent data.
- **Invitrogen™ instant ELISA kits**—the one-wash instant ELISA kit introduces fewer handling steps and reduces hands-on time to increase productivity.
- **Invitrogen™ phosphospecific ELISA kits**—measure phosphorylated proteins in cell lysates.
- **Antibody pair kits**—keep costs low with our affordable coat-it-yourself ELISA plate set. Each set contains the necessary reagents, including ELISA-optimized matched antibody pairs, standards, detection reagents, coating buffers, sample diluent, and tetramethylbenzidine (TMB) substrate solution. Plates are optional.

- **ProQuantum high-sensitivity (HS) assay kits**—take advantage of a platform innovation that provides researchers with an easy-to-run, high-performance assay, utilizing proximity-based amplification technology. This assay combines the analyte specificity of high-affinity antibody–antigen binding with the signal detection and amplification capabilities of real-time PCR to achieve highly sensitive protein quantitation, using very small sample volumes.
- **ProcartaPlex multiplex immunoassays**—quantitate more with less sample. ProcartaPlex multiplex immunoassays utilize Luminex® xMAP® technology for profiling up to 80 analytes in a single 25–50 µL sample.
- **QuantiGene assays**—measure gene expression directly from cell and whole blood lysates without RNA purification. QuantiGene assays are an accurate and precise method for single or multiplex gene expression quantitation.

Go to [thermofisher.com/immunoassays](https://thermofisher.com/immunoassays) to find:

- Our helpful immunoassay selection guide that allows you to search for assays based on your target protein
- Detailed information on all of our antibody pair kits, ELISA kits, ProQuantum high-sensitivity assay kits, and ProcartaPlex multiplex assay kits
- Important data demonstrating our assay specificity and sensitivity

Table 1. Components for different immunoassay kits.

	Type of immunoassay					
	Antibody pair ELISA kits	Coated ELISA kits	Instant ELISA kits	ProQuantum HS kits	ProcartaPlex assays (all platforms)	QuantiGene kits
96-well plate	Optional	•	•	NA	Flat bottom	Hybridization plates (and magnetic separation plates for multiplex)
Pretitrated capture antibody	•	Precoated	Precoated	•	Precoated capture bead	Precoated capture probe
Pretitrated detection antibody	•	•	•	•	•	Label probe
Recombinant protein standard	•	•	Additional well strips included	•	•	NA
Stop solution	Not included	•	•	NA	NA	NA
Substrate solution	•	•	•	NA	SA-PE	SA-PE (multiplex); alkaline phosphatase (singleplex)
Assay buffer	•	•	Sample diluent	•	•	•
Wash buffer	Not included	•	•	NA	•	•
Coating buffer	•	NA	NA	NA	NA	NA

**Note:** Our immunoassays are available in various kit sizes. Kits are calibrated to National Institute for Biological Standards and Control (NIBSC) or World Health Organization (WHO) standards (if available). SA = streptavidin, PE = phycoerythrin, NA = not applicable.



**Table 2. Characteristics of Invitrogen™ immunoassay kits.**

	Type of immunoassay						
	Build-it-yourself immunoassays	Uncoated ELISA and antibody pair kits	Coated ELISA kits*	Instant ELISA kits	ProQuantum HS kits	ProcartaPlex immunoassays**	QuantiGene kits
<b>Biomarker type</b>	Protein	Protein	Protein	Protein	Protein	Protein	RNA
<b>Ready-to-use reagents</b>	No; need overnight coating process	Yes; need overnight coating process	Yes	Yes	Yes	Yes	Yes
<b>Analytical sensitivity†</b>	<10 pg/mL	<10 pg/mL	<10 pg/mL	<5 pg/mL	<0.1 pg/mL	<10 pg/mL	200–1,000 RNA copies
<b>Dynamic range†</b>	<5–250 pg/mL	<5–500 pg/mL	<5–250 pg/mL	<7.8–500 pg/mL	0.064–5,000 pg/mL	<5–2,000 pg/mL	≤3 logarithmic units
<b>Sample volume</b>	10–100 µL	10–100 µL	10–100 µL	25–50 µL	2–5 µL	25–50 µL	20–80 µL
<b>Incubation time†</b>	4 hr	3–4.5 hr	2.5–4 hr	4 hr	2 hr	3.5 hr	Overnight
<b>Multiplexibility</b>	No	No	No	No	No	Yes	Yes
<b>Number of targets measured per well</b>	1	1	1	1	1	1–80	1–80
<b>Readout</b>	HRP-TMB (colorimetric)	HRP-TMB (colorimetric)	HRP-TMB (colorimetric)	HRP-TMB (colorimetric)	FAM™ (fluorescence)	R-PE (fluorescence)	R-PE (fluorescence for multiplex); Lumigen™ APS-5 (chemiluminescence for singleplex)
<b>Instrumentation needed</b>	Microplate reader	Microplate reader	Microplate reader	Microplate reader	qPCR instrument	Luminex® instrument	Luminex instrument (multiplex); microplate reader (singleplex)
<b>Instrument read time</b>	2 min	2 min	2 min	2 min	36–72 min	20–60 min	20–75 min

\* Values in this table refer to standard colorimetric kits. Ultrasensitive kits are also available.

\*\* Immunoassays for the Luminex platform.

† Every assay has its own specifications. Please consult the protocol for your specific immunoassays or kits.

### Benefits of precoated ELISA kits:

- **Robust**—low inter- and intra-assay coefficient of variation (CV)
- **Easy to use**—includes all required assay buffers and reagents, and a straightforward protocol
- **Rigorously verified**—optimized for serum, plasma, and cell culture supernatants

Request bulk packaging or lot reservation, or go custom at [thermofisher.com/iaspecialservices](https://www.thermofisher.com/iaspecialservices)

# Single-biomarker quantitation of protein targets



## Precoated ELISA kits

Our ELISA kits are developed to meet specifications typically expected by customers, including standard calibration, precision, sensitivity, specificity, recovery, lot-to-lot consistency, linearity, and parallelism (Table 3).

**Table 3. Rigorous assay verification of ELISA kits helps ensure consistent, reliable results.**

Specification	Description	What does it mean for you?
Standard calibration	Calibrated to NIBSC, if available	Allows accurate quantitation and consistent standard of reference across multiple platforms
Precision	Average inter-assay CV <10%; average intra-assay CV <10%	Consistent results
Sensitivity	Relevant levels of protein are detected for specific assay type	Enables detection of low levels of protein
Specificity	Cross-reactivity tests are performed with similar analytes	Helps to ensure accurate measurement of the protein of interest
Recovery	Buffers are optimized to minimize matrix effects	Helps to ensure accurate quantitation of samples within a complex matrix, including serum and plasma
Lot-to-lot consistency	In-house controls are tested to measure within set ranges	Helps to ensure consistent results with new lots
Linearity of dilution	Linear results over the quantitative range of the assay	Serial dilution of a sample is quantitated accurately
Parallelism	Recombinant protein standards mimic native proteins	Samples can be measured with confidence

## Coated ELISA kits that include ready-to-use reagents

### Quantitate with confidence

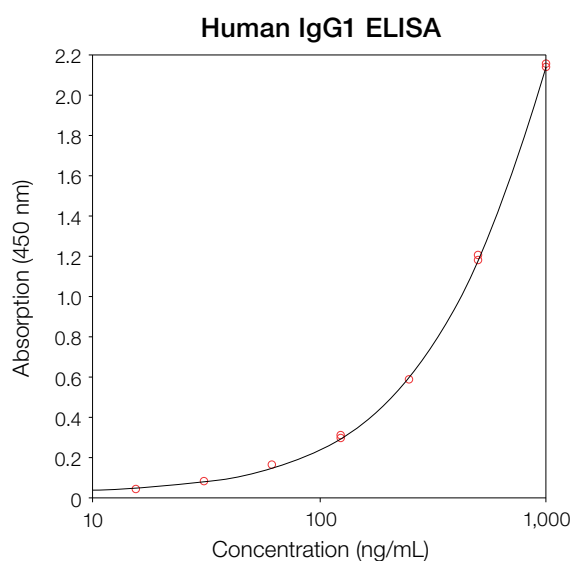
Coated ELISA kits for the quantitation of individual analytes from a variety of sample types help provide lower inter- and intra-assay variability. Developed and manufactured for labs that require verified and comprehensively tested kits, coated ELISA kits offer lot-to-lot consistency and help ensure the highest possible ELISA performance (see an example standard curve in Figure 5). Ready-to-use reagents, including precoated plates, help ensure consistent data throughout your research.

### Benefits of precoated ELISA kits:

- **Robust**—low inter- and intra-assay CV
- **Easy to use**—includes all required assay buffers and reagents, and a straightforward protocol
- **Rigorously verified**—optimized for serum, plasma, and cell culture supernatants

Request bulk packaging or lot reservation, or go custom at

[thermofisher.com/iaspecialservices](https://thermofisher.com/iaspecialservices)



**Figure 5. Standard curve for Invitrogen™ Human IgG1 ELISA Kit (Cat. No. BMS2092).**

## Instant ELISA: the one-wash ELISA kit

### Reduce steps and hands-on time by as much as 60%

The one-wash instant ELISA kit requires fewer handling steps than the conventional ELISA kit (Table 4). The instant ELISA kit contains all of the necessary components, including capture antibody and lyophilized detection antibody, streptavidin-HRP, and sample diluent.

Additionally, the strip wells containing the standards for the standard curve are provided separately; they are ready to use, helping save both time and wells. Less hands-on time can help increase productivity, which leaves more time for your research.

**Table 4. Steps for conventional ELISA vs. instant ELISA kits.**

17 steps for conventional ELISA		7 steps for instant ELISA kits	
1	Washing of coated plate		
2	Reconstitution of standard protein		
3	Addition of sample diluent to standard wells		
4	Titration of standard curve	1	Rehydration of plate
5	Addition of sample diluent	2	Addition of sample
6	Addition of sample	3	Incubation
7	Dilution of biotin conjugate		
8	Addition of biotin conjugate		
9	Incubation		
10	Preparation of streptavidin-HRP conjugate		
11	Washing step		
12	Addition of streptavidin-HRP conjugate		
13	Incubation		
14	Washing step	4	Washing step
15	Addition of TMB substrate	5	Addition of TMB substrate
16	Addition of stop solution	6	Addition of stop solution
17	Calculation of results	7	Calculation of results

### Benefits of instant ELISA kits:

- **Time saving**—only requires about 15 min for setup
- **Maximum accuracy**—no need to add antibody or perform serial dilution of standards; reduced handling can mean less error and more consistent results
- **Better value**—generate standard curve data in parallel with additional well strips provided, to enable use of all 96 wells for your samples

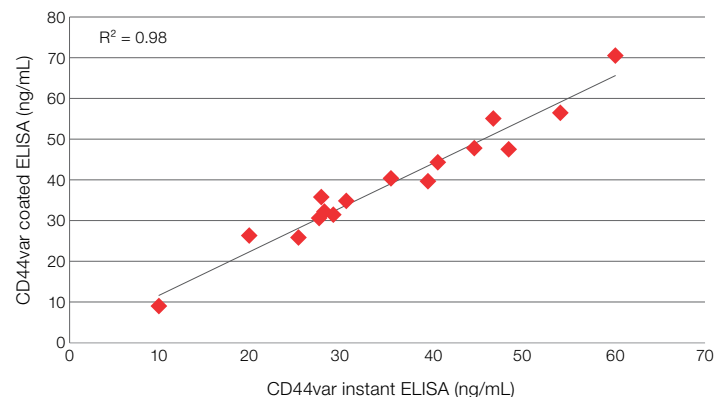
### Switching to instant ELISA kits

The instant ELISA kits utilize the same antibody pairs for many analytes across all assay formats. This enables cross-platform performance and comparable data using any of our instant ELISA kits, ELISAs, and ProcartaPlex multiplex assays. For example, if you are currently using an Invitrogen™ Human CD44var ELISA Kit, which includes a pre-coated microplate, you can be confident in switching to the Invitrogen™ Human CD44var Instant ELISA Kit, knowing that the data generated across the two kits will remain consistent (Figure 6).



Find out more about instant ELISA kits at

[thermofisher.com/instanelisa](https://thermofisher.com/instanelisa)



**Figure 6. Performance comparison of the human CD44var instant ELISA to the coated ELISA.** The Human CD44var Instant ELISA Kit (Cat. No. BMS220INST) was tested against the Human CD44var ELISA Kit (Cat. No. BMS220). A coefficient of determination ( $R^2$ ) of 0.98 was observed using 16 serum samples, demonstrating comparable performance of the instant ELISA and coated ELISA kits.

## High-sensitivity ELISA kits

Our Invitrogen™ high-sensitivity ELISA kits might be just what you need for those samples with low protein concentrations. You can measure sub-picomolar concentrations in serum and plasma for some of the most common proteins with highly variable biological concentrations.

Protein	Kit type	Sensitivity	Standard range	Cat. No.
Human IL-6	Standard ELISA	0.92 pg/mL	1.56–100 pg/mL	<a href="#">BMS213-2</a>
	High-sensitivity ELISA	0.03 pg/mL	0.8–5 pg/mL	<a href="#">BMS213HS</a>
Human IL-10	Standard ELISA	1 pg/mL	3.15–200 pg/mL	<a href="#">BMS215-2</a>
	High-sensitivity ELISA	0.05 pg/mL	0.39–25 pg/mL	<a href="#">BMS215HS</a>
Human IL-12p70	Standard ELISA	2.1 pg/mL	3.1–200 pg/mL	<a href="#">BMS238</a>
	High-sensitivity ELISA	0.1 pg/mL	0.16–10 pg/mL	<a href="#">BMS238HS</a>
Human IL-17A	Standard ELISA	0.5 pg/mL	1.6–100 pg/mL	<a href="#">BMS2017</a>
	High-sensitivity ELISA	0.01 pg/mL	0.23–15 pg/mL	<a href="#">BMS2017HS</a>
Human TNF alpha	Standard ELISA	5 pg/mL	23–1,500 pg/mL	<a href="#">BMS2034</a>
	High-sensitivity ELISA	0.13 pg/mL	0.31–20 pg/mL	<a href="#">BMS223HS</a>
Human IFN gamma	Standard ELISA	0.99 pg/mL	1.6–100 pg/mL	<a href="#">BMS228</a>
	High-sensitivity ELISA	0.06 pg/mL	0.16–10 pg/mL	<a href="#">BMS228HS</a>
Human IL-1 beta	Standard ELISA	0.3 pg/mL	3.9–250 pg/mL	<a href="#">BMS224-2</a>
	High-sensitivity ELISA	0.05 pg/mL	0.16–10 pg/mL	<a href="#">BMS224HS</a>
Human IL-2	Standard ELISA	9.1 pg/mL	18.8–1,200 pg/mL	<a href="#">BMS221-2</a>
	High-sensitivity ELISA	0.4 pg/mL	0.94–60 pg/mL	<a href="#">BMS221HS</a>



# Phosphospecific ELISA kits

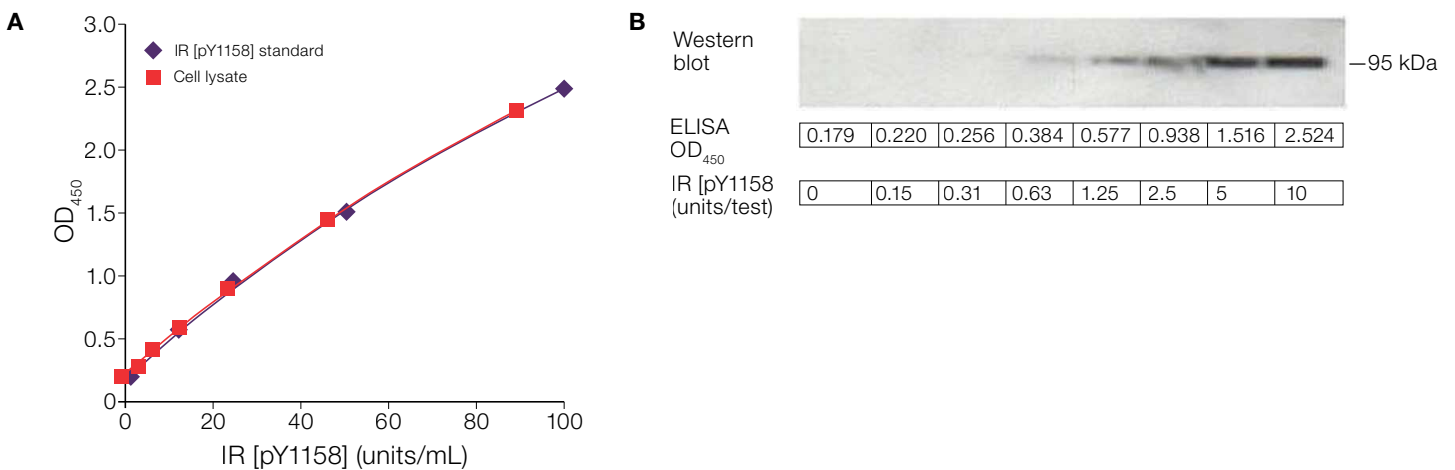
## Cell signaling assay

For studies of intracellular proteins involved in signaling, Invitrogen™ phosphospecific ELISA kits are available for measurement of total, and phosphorylated, modified, or cleavage site–specific proteins. Advantages and benefits of these kits include:

- **Specificity**—two antibodies directed against the analyte allow better specificity than western blotting
- **Sensitivity**—more sensitive than western blotting
- **Quantitation**—get quantitative data in contrast to western blots (Figure 7)
- **Medium throughput**—96-well format, results in 4 hours, and no densitometry analysis needed



Find out more about our phosphospecific ELISA kits at [thermofisher.com/phosphoelisas](https://thermofisher.com/phosphoelisas)



**Figure 7. Confirmation of western blotting data with quantitative results by phosphospecific ELISA assay. (A)** Representative standard curve for an insulin receptor (IR) [pY1158] phosphoELISA, with parallel titration of natural IR [pY1158] from insulin-stimulated CHO T cell lysates. **(B)** Representative data showing titration of IR [pY1158] in insulin-stimulated CHO T cell lysates as measured using the IR [pY1158] ELISA and western blot.

# Uncoated ELISA and antibody pair kits

Coat it yourself: optimized reagents all in one box



## Keep costs low

Uncoated ELISA and antibody pair kits are ideal for experienced ELISA users on a budget, who have less stringent requirements for inter- and intra-assay variance (Figure 8). Affordable ELISA kits using coat-it-yourself plates also include the necessary reagents to prepare and run an ELISA, including ELISA-optimized antibody pairs, standards, detection reagents, wash buffers, and coating buffers (plates are optional).

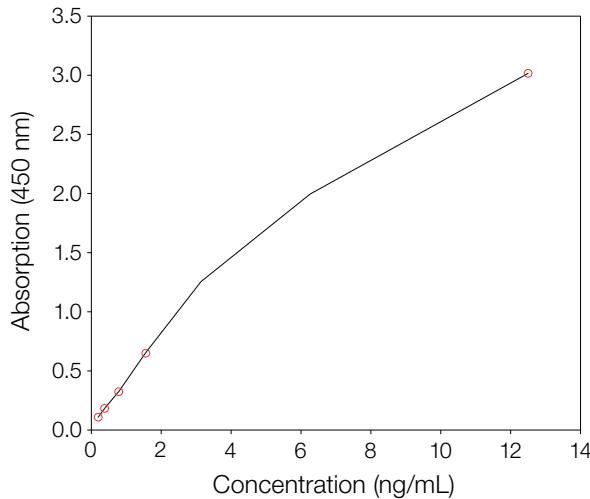


Figure 8. Representative standard curve for Invitrogen™ TGF beta-1 Mouse Uncoated ELISA Kit with plates (Cat. No. 88-50690).

## Benefits of uncoated ELISA and antibody pair kits:

- **Flexible**—purchase based on the volume of your assay through a choice of package sizes, including 2-, 10-, and 20-plate kits
- **Complete and easy to use**—includes optimized antibody pairs and recombinant standards, TMB substrate, and all essential reagents and buffers—unlike ELISA kits from other suppliers
- **Affordable**—priced to accommodate the most demanding budgets and to help maximize your research

## Using antibody pairs to make an ELISA

Watch our video at [thermofisher.com/antibodypairs](https://thermofisher.com/antibodypairs) to learn how to use antibody pairs to make an ELISA.



Find out more about uncoated ELISA kits at [thermofisher.com/uncoatedelisa](https://thermofisher.com/uncoatedelisa)

## ELISA validation and quality control

Validation and quality control are critical aspects for creating reliable ELISA kits. That's why we put our kits through rigorous testing to help ensure they work for you time and time again.

View the ELISA validation and QC testing guide at [thermofisher.com/elisavalidation](https://thermofisher.com/elisavalidation)



## ELISA troubleshooting resources

Is your ELISA not working? Invitrogen ELISA kits are built to be easy, but things can still go wrong. To help, we've put together a collection of troubleshooting resources.

Browse our selection of tips and tricks at [thermofisher.com/elisatroubleshooting](https://thermofisher.com/elisatroubleshooting)



## ELISA selection guide

With thousands of ELISA kits to choose from, we're confident you'll find the right kit for you.

Visit our selection guide to find your ELISA at [thermofisher.com/elisa](https://thermofisher.com/elisa)



# ProQuantum high-sensitivity immunoassay kits



The ProQuantum high-sensitivity immunoassay is a platform innovation that provides researchers with an easy-to-run, high-performance assay with no proprietary instrument to purchase. By utilizing proximity-based amplification technology, we have combined the analyte specificity of high-affinity antibody-antigen binding with the signal detection capabilities of real-time PCR, to achieve a highly sensitive protein quantitation assay with a large dynamic range. With its ability to detect even lower levels of protein than traditional methods with very small sample consumption, you can get the most out of your precious or limited samples.

## Here's how it works

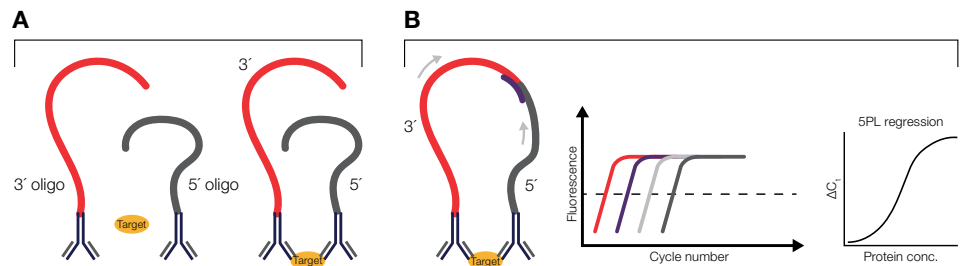
The ProQuantum immunoassays utilize a matched pair of target-specific antibodies each conjugated to a DNA oligonucleotide. During antibody-analyte binding, the two DNA oligos are brought into close proximity, which then allows for ligation of the two strands and subsequent creation of a template strand for amplification. This proximity-based technology leverages the sensitivity and large dynamic range of Applied Biosystems™ TaqMan™ real-time PCR technology (Figure 9).



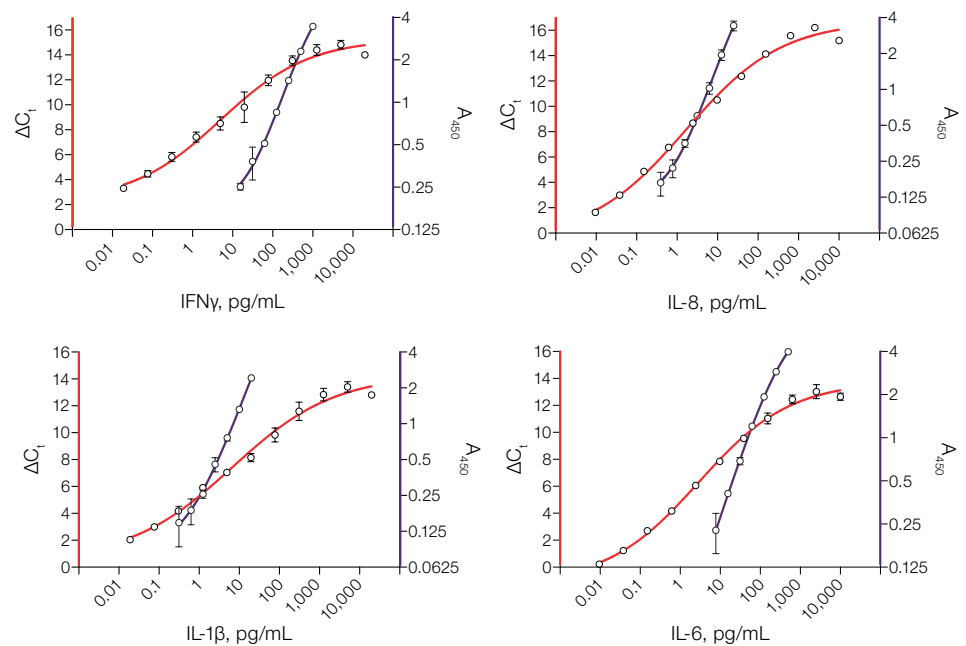
Find out more and select your assay at [thermofisher.com/proquantum](https://thermofisher.com/proquantum)

## The benefits of ProQuantum immunoassays include:

- **High-sensitivity**—detect low levels of proteins with greater sensitivity than traditional ELISA methods
- **Broad dynamic range**—≥5 logarithmic units, minimizing sample dilutions to help ensure they fall within the range (Figure 10)
- **Small sample consumption**—use 2–5  $\mu\text{L}$  of sample (for example, 2  $\mu\text{L}$  vs. 75  $\mu\text{L}$  for triplicate wells with other methods)
- **Fast, easy workflow**—about 2 hr from sample to answer, no wash steps, and a single 1 hr incubation
- **No proprietary instrument to purchase**—runs on any real-time PCR instrument
- **Includes intuitive analysis software**—comprehensive data analysis and statistical groupwise comparison



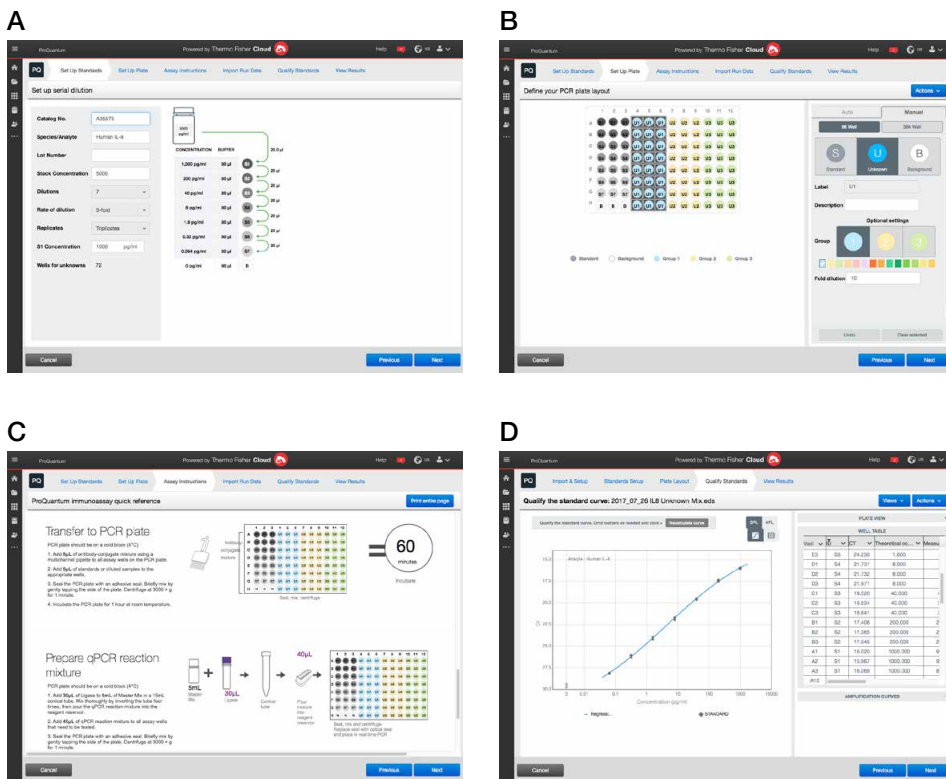
**Figure 9. How ProQuantum immunoassay works.** (A) Antibody conjugates binding to target (1 hr incubation). (B) Ligation and amplification of signal (in qPCR instrument).



**Figure 10. Superior sensitivity and broader dynamic range.** Standard curves for Invitrogen™ ProQuantum™ High-Sensitivity Human IFN $\gamma$ , IL-8, IL-1 $\beta$ , and IL-6 immunoassay kits (red) show larger dynamic range and improved separation at the low end of the curve for greater sensitivity than normal and ultrasensitive ELISAs (purple).

## Data analysis using ProQuantum software

The intuitive Invitrogen™ ProQuantum™ cloud-based or desktop software is available to support your data analysis. This step-by-step software helps you set up your assay, including help with the standard curve dilution calculation and plate layout for 96 or 384 wells, and provides customized lab bench instructions (cloud-enabled version only) (Figure 11). Once the assay has been performed, simply upload your run files from the qPCR instrument (see qPCR options on [page 38](#)) to the ProQuantum Software and analyze. Analysis features include standard curve qualification, outlier detection, 4PL or 5PL regression fit, results for unknown samples in pg/mL, and groupwise statistics. Easily access and securely share results with colleagues anywhere, anytime with our cloud-based Thermo Fisher™ Connect Platform. Access free ProQuantum cloud-enabled software at [apps.thermofisher.com/apps/proquantum](https://apps.thermofisher.com/apps/proquantum). A desktop version is now available (Cat. No. A43594).



**Green benefits**

- Less waste, use of fewer resources: 47–59% less plastic waste generated, 230 mL less water used per assay
- Sustainable packaging: 84% less packaging material

**Figure 11. Assay setup and analysis using ProQuantum Software.** (A) Standard curve setup. (B) Plate setup. (C) Customized lab instructions. (D) Data and results.

**Need a custom ProQuantum assay? Lot reservation? Special bulk packaging?**  
 We provide services for custom target development of your chosen protein targets and immunoassay platform, as well as lot reservations and custom packaging for bulk orders.  
 Find out more at [thermofisher.com/iaspecialservices](https://thermofisher.com/iaspecialservices)



# Single-biomarker quantitation of mRNA targets

## QuantiGene Singleplex kits

Accurately measure single RNA transcripts or DNA copy number using a standard luminometer and the Invitrogen™ QuantiGene™ Plex Gene Expression Assay with the Invitrogen™ QuantiGene™ Plex Assay Kit. Use one of thousands of probe sets available or design your own to target custom sequences. QuantiGene RNA assays measure gene expression directly from cell and whole blood lysates or tissue homogenates without the need to purify or amplify RNA. Direct measurement is possible because target RNAs are captured through probe hybridization and quantified through branched DNA technology that amplifies the signal. The signal is read using a standard luminometer for single targets.

### Highlights and key advantages of the QuantiGene assay:

- **Quantitate directly from crude samples**—no need for RNA extraction, cDNA synthesis, or PCR amplification
- **Works with difficult sample types**—works with degraded and crosslinked RNA in formalin-fixed, paraffin-embedded (FFPE) tissues, with fresh or frozen tissues (animal or plant), whole blood, cultured cells, bacteria, and viruses
- **Simple workflow**—lyse-and-go sample preparation, easily automatable ELISA-like protocol, and readout using any standard luminometer
- **Large inventory of validated genes**—select from over 22,500 genes to create pathways and disease-themed panels for QuantiGene Plex assays
- **Standardized formats to enable easy scale-up**—96-well plate, 384-well plate, and plex-in-plex formats available

### Branched DNA technology

QuantiGene Plex and Singleplex assays utilize branched DNA (bDNA) technology. bDNA technology uses sequential nucleic acid hybridization for a unique approach to RNA and DNA quantification by amplifying a reporter signal rather than the template. This method measures the transcripts at physiological levels.

After cells are lysed or tissue samples homogenized, a pair of target-specific probe sets (target probe) hybridizes to contiguous sequences on the target RNA (Figure 12). Signal amplification is achieved through successive hybridization of short oligonucleotide sequences to build the bDNA structure (bDNA “tree”), formed by pre-amplifier, amplifier, and label probe oligos, resulting in excellent specificity, low background, and a high signal-to-noise ratio. Addition of a chemiluminescent substrate (singleplex assays) or fluorescent reporter (multiplex assays) generates a signal directly proportional to the amount of target RNA or DNA present in the sample.

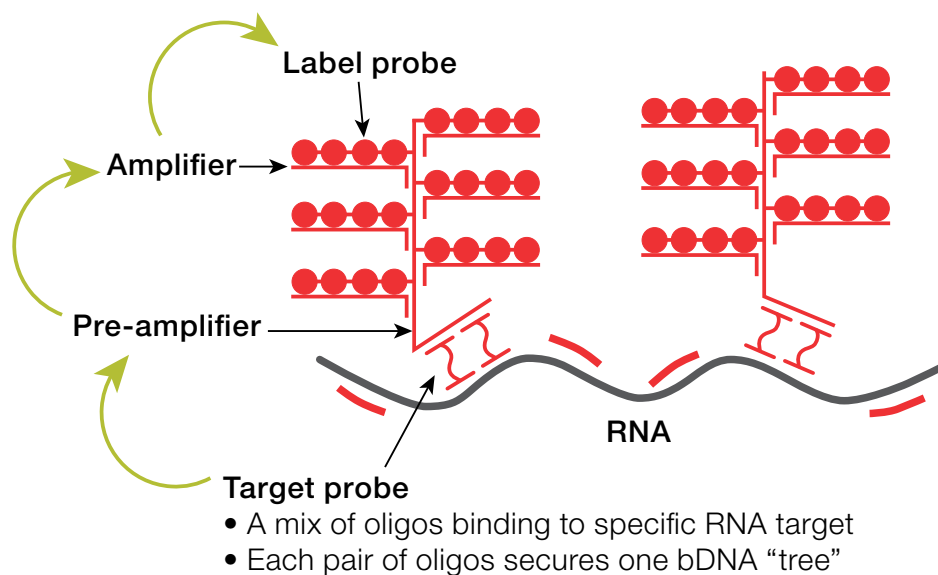


Figure 12. Overview of bDNA signal amplification.



To learn more about QuantiGene assays and search our catalog of over 22,500 genes, visit [thermofisher.com/quantigene](https://thermofisher.com/quantigene)

# Accessory reagents and equipment

Thermo Fisher Scientific offers a comprehensive line of accessory reagents and equipment to support your immunoassay needs.

Reagents include:

- Primary antibodies
- Secondary antibody conjugates
- Coated microplates
- Blocking buffers
- Diluents
- Substrates

See a complete listing of accessory reagents for ELISAs at [thermofisher.com/elisaaccessories](https://www.thermofisher.com/elisaaccessories)

## Accessory equipment includes:

- Thermo Scientific™ Multidrop™ reagent dispensers
- Centrifuges
- Cold storage equipment
- Single-channel pipettes
- Multichannel pipettes
- Pipette tips
- Plate washers
- Plate readers and analysis software

Get a comprehensive overview of immunoassay equipment offerings at [thermofisher.com/elisainstrumentsandequipment](https://www.thermofisher.com/elisainstrumentsandequipment)

## Microplate washers

### Wellwash Microplate Washer

Wash 96-well plates securely with the Thermo Scientific™ Wellwash™ Microplate Washer, an easy-to-use, reliable microplate-strip washer for routine ELISA applications.

The Wellwash system features a graphical user interface, local language versions, and USB port for optimal ease of use and convenience.



### Wellwash Versa Microplate Washer

Wash 96- or 384-well plates securely with the Thermo Scientific™ Wellwash™ Versa Microplate Washer, an easy-to-use microplate-strip washer for routine ELISA applications. Designed for high performance and versatility, enjoy

the ease of use and convenience provided through a graphical interface, local language versions, and USB port.



See more at [thermofisher.com/wellwash](https://www.thermofisher.com/wellwash)

Considerations	Wellwash washer	Wellwash Versa washer
Languages	9	9
Plate types	96-well	96- and 384-well
Wash heads (depending on model)	1 x 8 or 1 x 12	Two 1 x 8 or two 1 x 12
Optional wash heads	1 x 12 or 1 x 8	Optional two 1 x 12 or two 1 x 8; optional 1 x 16 (for 384-well plate); optional 2 x 8 (for 96-well plate)
Wash bottles	One 2 L	Two 2 L
Rinse bottle	No	One 2 L
Waste bottle	One 2 L	One 4 L
USB port for protocol import and export	Yes	Yes
Aerosol cover	Yes	Yes
Robot compatibility	No	Yes
IQ/OQ/IPV	Yes	Yes

# Microplate readers

## Multiskan FC Microplate Photometer

The Thermo Scientific™ Multiskan™ FC Microplate Photometer is a reliable and robust microplate photometer designed for a wide variety of applications from ELISAs to enzyme kinetic studies.



It offers fast and accurate measurements enabling complete 96-well plate reading in less than 7 seconds.

- Can be used as a stand-alone instrument or under PC control with intuitive Thermo Scientific™ SkanIt™ Software
- Qualitative and quantitative calculations and assay QC available for both user interfaces
- Shaking and optional incubation up to 50°C for temperature-critical assays
- Robot compatibility for high-throughput environments

Find out more about our microplate readers at [thermofisher.com/platereaders](http://thermofisher.com/platereaders)

## Varioskan LUX Multimode Microplate Reader

Designed for bioscience researchers with a wide variety of needs, the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader provides a flexible range of measurement modes. The instrument simplifies measurement setup with automatic dynamic range selection; its smart safety controls ease your workflow to help you avoid experimental errors. The Varioskan LUX multimode reader offers you:



- Versatility to run assays for photometric and fluorometric quantitation of proteins and RNA
- Five measurement modes: endpoint, kinetic, spectral, multipoint, and kinetic spectra
- Spectral scanning for assay optimization
- Modular, upgradable system for customization

SkanIt Software provides unlimited licenses with instrument purchase and ready-made sessions, available for running assays immediately. It is the same intuitive software that drives all Thermo Scientific™ microplate readers.

Find out more at [thermofisher.com/skanit](http://thermofisher.com/skanit)

Considerations	Multiskan FC Microplate Photometer	Varioskan LUX Multimode Microplate Reader
Wavelength range (nm)	340–850	Fluorescence excitation and absorbance: 200–1,000 nm Fluorescence emission: 270–840 nm Luminescence: 360–670 nm (spectral scanning: 270–840 nm) TRF excitation: fixed to 334 nm (spectral scanning 200–840 nm) TRF emission: 400–670 nm (spectral scanning 270–840 nm) Excitation for AlphaScreen™ assays: fixed to 680 nm Emission for AlphaScreen assays: 400–660 nm
Wavelength selection	Filters	Monochromator Filters for luminescence, TRF, and AlphaScreen tools
Wavelength scanning	No	Yes
Plate format	96- to 384-well	6- to 1,536-well (fluorometry, TRF, luminometry, AlphaScreen tool) 6- to 384-well (absorbance)
Incubation	Yes	Yes
Shaking	Yes	Yes
Dispensers	No	One or two (optional)
Top or bottom reading	Not applicable	Top or bottom (optional)
Cuvettes	No	No
Thermo Scientific™ $\mu$ Drop™ Plates	No	Yes
Gas control module	No	Yes (optional)
Onboard software	Yes	No
PC software	SkanIt Software (unlimited licenses)	SkanIt Software (unlimited licenses)
Robot compatibility	Yes	Yes
Applications	Immunoassays (ELISA); colorimetric protein assays; cell proliferation, viability, and cytotoxicity assays; enzymatic activity assays; kinetic assays; endotoxin assays; bacterial growth assays	Immunoassays, including ELISA; protein assays (Invitrogen™ Quant-iT™ assay, direct quantitation, and colorimetric assays); nucleic acid (DNA, RNA) quantitation (Quant-iT and direct assays); cell proliferation, viability, and cytotoxicity assays; enzymatic activity assays; luciferase assays; calcium assays; FRET, BRET, TR-FRET, AlphaScreen, and AlphaLISA™ assays; spectral and kinetic assays; live-cell assays with integrated gas control module; QuantiGene Singleplex assays

# Multiplex biomarker quantitation: product overview

## Assays and instruments for protein and RNA targets

### Protein multiplex immunoassays

Quantitate up to 80 analytes in only 25  $\mu$ L of serum or plasma, or 50  $\mu$ L of cell culture supernatant with ProcartaPlex multiplex assays.

### Gene expression multiplex assays

Measure up to 80 RNA transcripts in a single well with QuantiGene Plex assays.

### Luminex instruments for target quantitation

Luminex xMAP technology combines advanced fluidics, optics, and digital signal processing with fluorescently dyed microspheres to enable the quantitation of multiple protein or nucleic acid targets from a single sample. ProcartaPlex multiplex immunoassays are designed for measurement of protein expression, and QuantiGene Plex assays are available for nucleic acid quantitation.

### High-throughput immunoassays and gene expression analysis—all from one supplier, on one instrument

As a partner of Luminex, Thermo Fisher Scientific has been providing Luminex platform users with a comprehensive offering of instruments and multiplex reagents for over 20 years. ProcartaPlex and QuantiGene Plex assays are available in 96-well and 384-well formats for high-throughput analysis. See [page 40](#) for more on high-throughput analysis options.

### ProcartaPlex multiplex immunoassays

ProcartaPlex multiplex immunoassays are bead-based assays for protein detection using Luminex xMAP technology. ProcartaPlex assays are based on the principles of a sandwich ELISA, using two highly specific antibodies binding to different epitopes of one protein. Invitrogen™ ProcartaPlex™ Simplex kits and panels can be easily combined to create your own panels, or we can mix and validate a panel for you.

### QuantiGene Plex assays for RNA quantitation

The QuantiGene Plex assay also utilizes Luminex xMAP technology and is a higher-throughput solution for multiplex analyses of gene expression, allowing researchers to measure up to 80 genes in a single well. This assay is fast, easy, and PCR-free—no RNA purification is needed. Additionally, custom-made panels can be designed, validated, and shipped typically within two to three weeks of a request.

### Why use Luminex technology?

Profile more biomarkers with less starting sample: quantitate up to 80 analytes simultaneously with the INTELLIFLEX™, FLEXMAP 3D®, or Luminex® 200™ instrument, and up to 50 analytes with the MAGPIX® instrument. All of our ProcartaPlex assays are compatible with as little as 25  $\mu$ L of plasma or serum, or 50  $\mu$ L of cell culture supernatant.

### Multiomics

Perform multiomics studies using a proven technology on a trusted platform. Hundreds of peer-reviewed publications have cited ProcartaPlex and QuantiGene Plex assays. You can have the advantage of looking at both genomics and proteomics all on one validated instrument platform.

### Comparison of different multiplex assay platforms

Assays	Analyte measured	Plex level	Targets available	Custom designs
ProcartaPlex multiplex immunoassays	Proteins	80	>500	Yes
QuantiGene Plex RNA assays	RNA	80	>22,500	Yes

# Multiplex biomarker quantitation of protein targets

## Luminex xMAP technology

### Quick, cost-effective, and accurate multi-analyte profiling system

The open-architecture of Luminex xMAP technology uses flow cytometry, microspheres, lasers, digital signal processing, and traditional chemistry—combining proven technologies in a unique way.

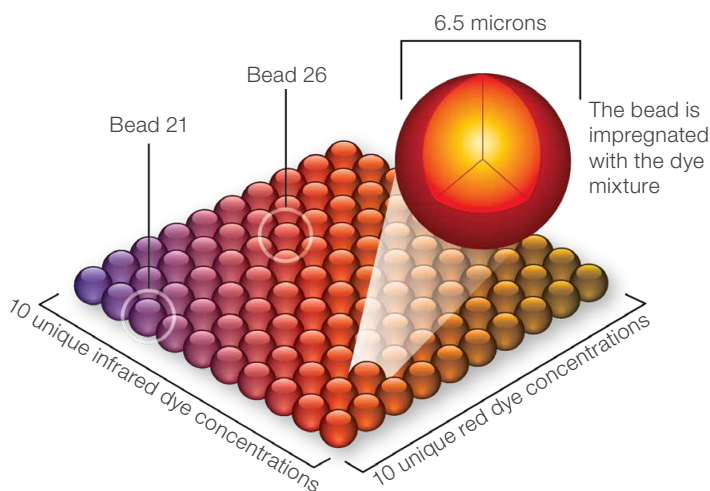
### Advantages

- Helps reduce costs and labor through multiplexing
- Smaller sample size requirements compared to single-result assays
- Enables fast, reproducible results from favorable kinetics of liquid bead array approach
- Broad coverage of applications, including protein expression and gene expression, to satisfy the need to understand both proteomic and genomic profiles

### How Luminex xMAP technology works

Sets of magnetic microspheres or beads are color-coded with combinations of red and infrared fluorophores. Each bead is coated with a reagent specific to a particular biomarker, allowing the capture and detection of several specific biomarkers from a single sample. Lasers in the compact analyzer excite the internal dyes that identify each bead as well as any reporter dye captured, providing quantitation of individual analytes on each bead. The analyzer reads many beads from each set, enabling rapid and precise results for several targets within a single sample.

ProcartaPlex assays have adopted the Luminex xMAP magnetic bead technology that uses Luminex® MagPlex® superparamagnetic 6.5-micron microspheres with a magnetic core. The beads are internally dyed with precise proportions of red and infrared fluorophores. Varying proportions of the red and infrared fluorophores result in 100 spectrally unique microspheres, which are identified by the Luminex xMAP detection systems. The conjugation of a distinct monoclonal antibody to a distinct bead allows for analysis of multiple analytes in a single well.



Find out which instrument is best for you and request a quote at [thermofisher.com/luminexinstruments](https://www.thermofisher.com/luminexinstruments)

# ProcartaPlex multiplex immunoassay kits

## Profile more biomarkers with less sample



ProcartaPlex multiplex immunoassays use the Luminex xMAP (multi-analyte profiling) technology that enables the simultaneous detection and quantitation of up to 80 protein targets in a single 25–50  $\mu$ L sample—from plasma, serum, cell culture supernatants, and other bodily fluids. The technology employs the use of differentially dyed capture beads for each target in a multiplex “ELISA-like” assay. ProcartaPlex immunoassays can profile up to 80 times more analytes using significantly less sample in the same time that it takes to perform a traditional sandwich ELISA.

ProcartaPlex multiplex immunoassays are available in off-the-shelf kits or you can create your own unique panel through our mix-and-match panel offering. It is often advantageous to start with a large panel and then reduce the number of biomarkers analyzed in a more focused panel. Whatever option works best for you, ProcartaPlex panels enable reproducible results throughout the course of your study.

### Features of ProcartaPlex panels

- More than 90% of ProcartaPlex assays can be combined with one another
- All ProcartaPlex assays are individually tested for specificity and endogenous, native protein detection
- Multi-analyte detection with a large dynamic range
- Scalable and reproducible performance regardless of plex size
- Largest multiplex panel on the market for quantitation of up to 80 analytes

ProcartaPlex multiplex immunoassays are available in multiple formats across six species (human, mouse, rat, nonhuman primate, porcine, and canine) to meet the needs of your research.

Get information regarding characteristic assay details and individual analytes at [thermofisher.com/procartaplex](https://thermofisher.com/procartaplex)

### ProcartaPlex multiplex assay options

Name	Description	Mixing required	Bead type	Base kit included
<b>ProcartaPlex Preconfigured panels</b>	Predefined, biologically relevant, and disease-defined panels using magnetic beads for the quantitative multiplex analysis of up to 80 analytes in a single sample; optimal performance and reproducibility through extensive testing for combinability, cross-reactivity, and interference	No	Magnetic	Yes
<b>ProcartaPlex Mix and Match panels</b>	Custom-blended and optimized panels deliver results tailored to the panel design of your choice. Simply provide your desired species, sample type, and instrument for use. Then select your desired analytes, and we will build and optimize a custom assay kit according to your specifications.	No	Magnetic	Yes
<b>ProcartaPlex Simplex kits</b>	Bead sets for the detection of individual analytes are designed to be added to ProcartaPlex panels for increased customization. Alternatively, multiple ProcartaPlex Simplex bead sets can be combined and run using the Invitrogen™ ProcartaPlex™ Basic Kit, which includes all non-target-specific reagents needed to perform the assay.	Yes	Magnetic	No

Have technical questions? Need help getting started? Email [luminexfas@thermofisher.com](mailto:luminexfas@thermofisher.com) to get a one-on-one technical consultation.

## Why ProcartaPlex assays?

### Flexibility

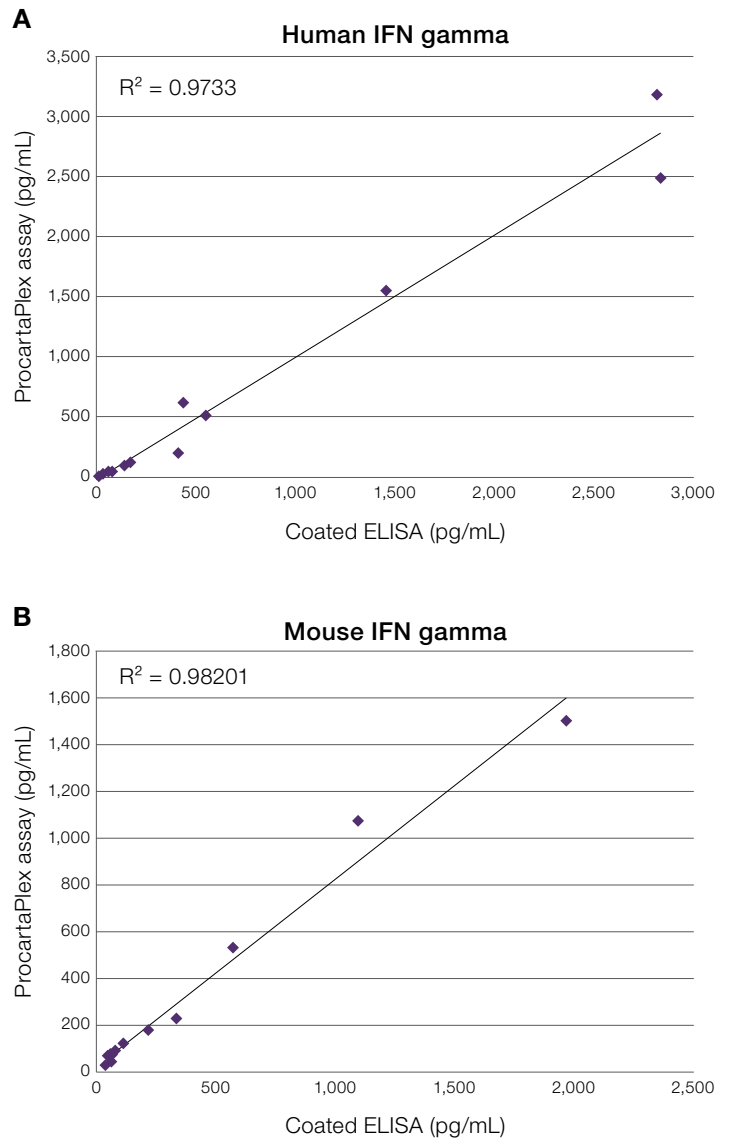
Get exactly the panel you want. Since more than 90% of ProcartaPlex assays can be combined with one another, ProcartaPlex assays allow the creation of highly specific as well as high-plex panels. We currently offer the largest published panel with a 65-plex assay [4,8-10].

### Reproducible results

ProcartaPlex assays are based on the principles of sandwich ELISA, where two highly specific antibodies recognize different epitopes on the same protein. Reproducible results come from reliable manufacturing. We own and operate a world-class hybridoma facility for manufacturing the antibodies that are used in ProcartaPlex assays, enabling the delivery of consistent, reproducible, and high-quality assays.

### Correlation to ELISA

Switch easily from ProcartaPlex assays to ELISA and vice versa with reliable results. Most of our ProcartaPlex assays use the same antibody pairs as our traditional plate-based ELISAs, resulting in a high correlation ( $R^2 > 0.9$ ) between the two assays. As shown in Figure 13, equivalent results ( $R^2 > 0.9$ ) were obtained using ProcartaPlex multiplex immunoassays and our traditional plate-based ELISAs for human (Cat. No. BMS228) and mouse (Cat. No. BMS606) IFN gamma. ProcartaPlex assays achieve quantitative results comparable to traditional ELISA results with the added benefits of reduced sample volume, shorter assay time, and lower cost.



**Figure 13. Results of an experiment measuring IFN gamma in stimulated human and mouse PBMCs.** Supernatants were serially diluted two-fold in normal human or mouse serum, to allow for differential cytokine levels in a serum matrix. Additional cytokines for human and mouse analytes were compared including IL-1, IL-17A, and TNF alpha, and similar performance was obtained (data not shown). PBMC = peripheral blood mononuclear cell

## Scalability

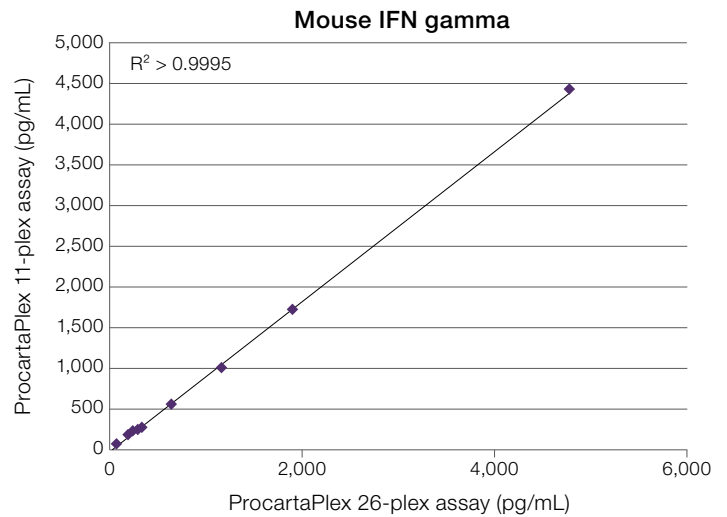
The ability to scale the number of analytes investigated in multiplex experiments is critical for the progression of many projects. For example, it is not uncommon to begin a project by analyzing a large, comprehensive 34-plex cytokine panel on fewer samples (i.e., 100) to determine which analytes are affected by a particular disease or drug treatment. The resulting list of analytes can then be investigated further, for instance, using an 11-plex panel, on a larger set of samples. Thus, there is a great need for scalability in multiplex panel assays in order to enable researchers to correlate all their data across different stages of their investigation. Many commercially available multiplex assays have not been developed with this level of consistency in mind. However, this specification is a key design requirement incorporated during ProcartaPlex assay development. Data in Figures 14 and 15 illustrate the high correlation between similar analytes in a large and small panel and the scalability when using ProcartaPlex assays. Similar results are obtained when investigating other analytes.

## Protein quantitation data analysis using ProcartaPlex analysis app

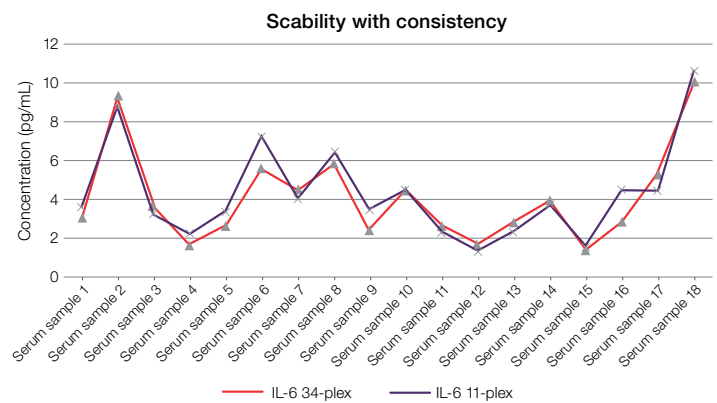
The intuitive Invitrogen™ ProcartaPlex™ Analysis App is a free software application available through the cloud-based Connect Platform that enables the analysis of ProcartaPlex immunoassay data. The software features include standard curve qualification, with built-in outlier detection, interpolation of unknown samples for quantitation, and group-wise statistical analysis.

### Features:

- **Minimized manual data entry**—assign lot-specific assay information to your raw data
- **Advanced analysis**—4-parameter logistic (4PL) or 5PL curve fit optimization, group-wise statistical and heat map analysis
- **Publication-ready data**—users can export detailed reports including images for presentations and publications
- **Easily see results**—view unknown sample concentrations from standard curve graphs or in table format
- **Compatible with 96- and 384-well formats**—simply import data from instrument platform





**Figure 14. Data illustrate the high correlation between large- and small-scale multiplex assays when using the ProcartaPlex assays.** Regression analysis comparing the Invitrogen™ ProcartaPlex™ Mouse Cytokine & Chemokine Panel 1 (26-plex) and the Invitrogen™ ProcartaPlex™ Mouse Th1/Th2 Cytokine Panel (11-plex) yielded an  $R^2$  value  $>0.9995$ .



**Figure 15. Scalability of ProcartaPlex multiplex immunoassays.** Human serum samples were run in parallel using both the Invitrogen™ ProcartaPlex™ Human Cytokine & Chemokine Panel 1A (34-plex) and the Invitrogen™ ProcartaPlex™ Human Th1/Th2 Cytokine Panel (11-plex). Experiments demonstrate consistency was achieved in both large- and small-scale multiplex panels.

Learn more:  
[thermofisher.com/procartaplex](https://thermofisher.com/procartaplex)

Access now:





## Product highlight: ProcartaPlex Human Immune Response Panel 80-Plex

The Invitrogen™ ProcartaPlex™ Human Immune Response Panel 80-Plex enables the study of immune function by analyzing 80 protein targets in a single well using Luminex xMAP technology. Analyze 80 cytokine, chemokine, and growth factor targets simultaneously for efficient immune response profiling, biomarker discovery, and validation. The panel is provided in a ready-to-use format with individual vials of capture and detection reagents formulated at 1X concentration, requiring less pipetting and experimental setup.

### ProcartaPlex panel content

ProcartaPlex Human Immune Response Panel 80-Plex							
Cat. No. EPX800-10080-901							
APRIL	CD40L	GM-CSF	IL-15	IL-23	IL-7	M-CSF	PTX3
BAFF	CXCL6 (GCP-2)	Granzyme A	IL-16	IL-27	IL-8	MDC	SCF
BLC	ENA-78	Granzyme B	IL-17A	IL-2R	IL-9	MIF	TNF beta
bNGF	Eotaxin	Gro alpha	IL-18	IL-3	IP-10	MIG	TNF alpha
CCL1 (I-309)	Eotaxin-2	HGF	IL-1 alpha	IL-31	I-TAC	MIP-1 alpha	TNF-R2
CCL17 (TARC)	Eotaxin-3	IFN alpha	IL-1 beta	IL-34	LIF	MIP-1 beta	TRAIL
CCL21 (6CKine/SLC)	FGF-2	IFN gamma	IL-2	IL-37	MCP-1	MIP-2 alpha (CXCL2)	TREM-1
CCL23 (MPIF)	Fractalkine	IL-10	IL-20	IL-4	MCP-2	MIP-3 beta (CCL19)	TSLP
CCL25 (TECK)	Gal-3	IL-12p70	IL-21	IL-5	MCP-3	MIP-3 alpha	TWEAK
CD30	G-CSF	IL-13	IL-22	IL-6	MCP-4 (CCL13)	MMP-1	VEGF-A

# Product highlight: ProcartaPlex hallmarks of cancer immunoassays

Discover a complete collection of ProcartaPlex panels to address the hallmarks of cancer, including cell proliferation and metastasis, resisting cell death, evading immune destruction, immune checkpoint inhibitors, and even exosome characterization (Figures 16 and 17). This expanded menu for detection of protein markers offers the largest selection and flexibility to customize panels of choice for extensive interrogation of cancer biology. These new panels can also be combined with existing markers involved in immuno-oncology, angiogenesis, and apoptosis, providing unique solutions for biomarker profiling in cancer research.

Visit [thermofisher.com/cancerhallmarks](https://thermofisher.com/cancerhallmarks) to explore the ProcartaPlex hallmarks of cancer collection of panels.

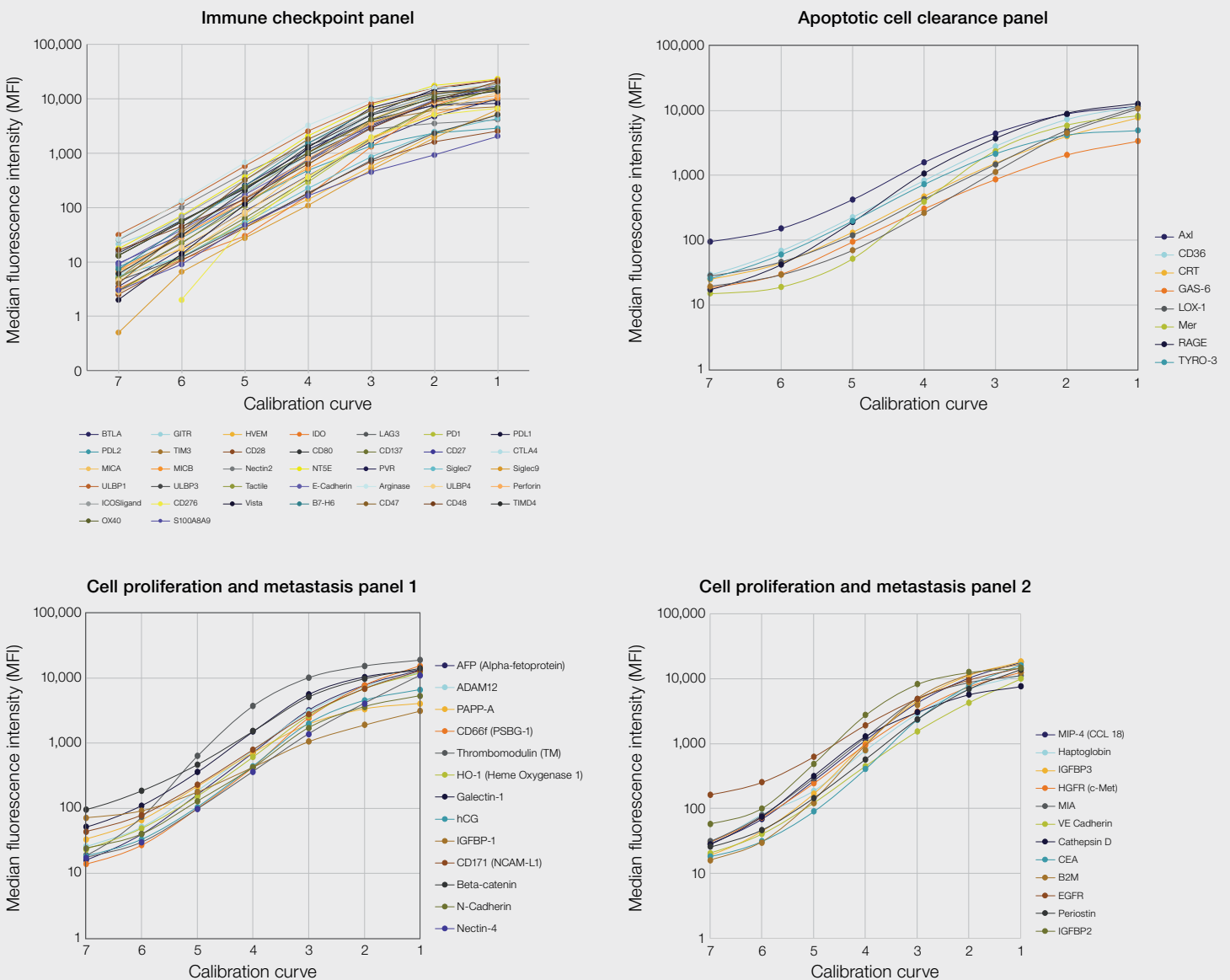
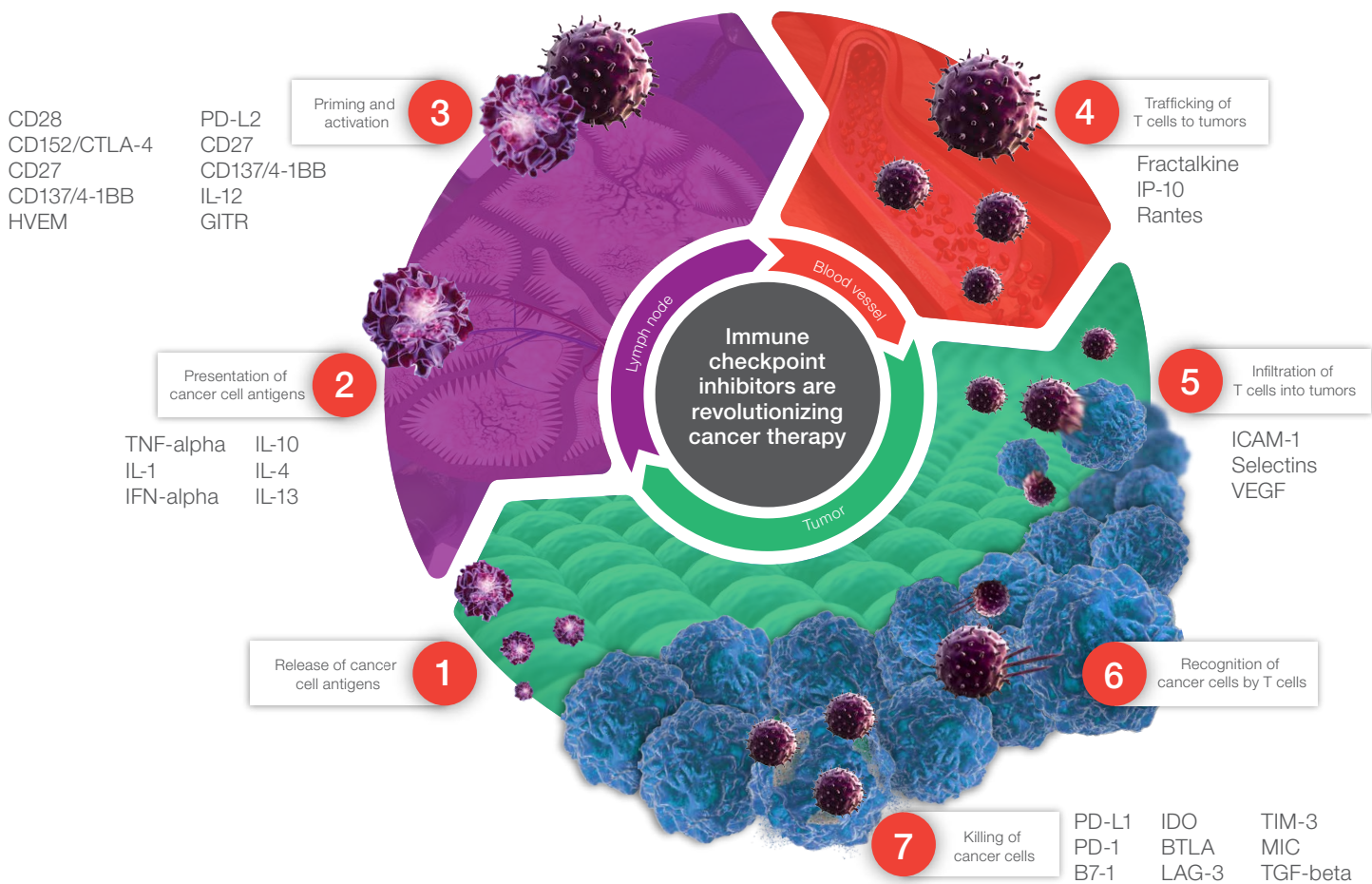


Figure 16. Standard curve examples from some of the ProcartaPlex panels demonstrating the high-level multiplex screening in one run of the assay to save time, sample, and labor.



**Figure 17. The cancer immunity cycle.** Immune checkpoint inhibitors are targets for biotherapeutic antibodies.

# ProcartaPlex high-sensitivity immunoassay kits



Invitrogen™ ProcartaPlex™ high-sensitivity immunoassay kits offer accurate quantitation of key cytokines significantly below the detection limit of conventional Luminex assays.

## Features of ProcartaPlex high-sensitivity immunoassay kits

- Highest sensitivity detection of cytokine concentrations, in fg/mL
- Inter- and intra-assay CV <10%
- Small sample input (25 µL) ideal for precious serum or plasma samples
- Easy-to-use, simple ELISA-like protocol completed in less than 4 hr
- Convenient, ready-to-use kits for quantitation of up to 9 cytokines
- Trusted platform based on the principles of a sandwich ELISA and utilizing Luminex xMAP technology

The ProcartaPlex high-sensitivity immunoassays enable the multiplexed analysis of human and mouse analytes in very low concentrations in various matrices, including serum, plasma, bodily fluids, and cell culture supernatant samples. These high-sensitivity multiplex assays are ideal when sample input is limited, and protein expression is in the low pg/mL range.

Accurate quantitation below conventional limits is achieved through a proprietary signal amplification technology. The signal amplification has negligible effects on the assay background, thereby increasing the signal-to-noise ratio of the cytokine standards on the lower end of the standard curve. This also allows for more accurate and reproducible measurement of cytokines with low expression levels.

## ProcartaPlex high-sensitivity panel

ProcartaPlex Human High-Sensitivity Panel 9-Plex		
Cat. No. EPXS090-12199-901		
IFN-gamma	IL-4	IL-12 p70
IL-1 beta	IL-6	IL-17A (CTLA-8)
IL-2	IL-10	TNF-alpha

## Assay specifications and data

Measure low-abundance cytokines with our ProcartaPlex high-sensitivity immunoassays. We offer simplex kits and panels for human and mouse cytokines. Find the most current offering of our available high-sensitivity assays at [thermofisher.com/highsensitivityprocarta](https://thermofisher.com/highsensitivityprocarta).

## ProcartaPlex Simplex human high-sensitivity kits

Cat. No.	Analyte	ULOQ* (pg/mL)	LLOQ** (pg/mL)	Inter-assay CV	Intra-assay CV
EPXS010-10283-901	GM-CSF	7,330	1.8	<10	<10
EPXS010-10228-901	IFN-γ	1,250	0.31	<10	<10
EPXS010-10224-901	IL-1 β	1,330	0.32	<10	<10
EPXS010-10221-901	IL-2	1,820	0.44	<10	<10
EPXS010-10225-901	IL-4	3,530	0.86	<10	<10
EPXS010-10278-901	IL-5	2,720	0.66	<10	<10
EPXS010-10213-901	IL-6	4,230	1.03	<10	<10
EPXS010-10204-901	IL-8	890	0.22	<10	<10
EPXS010-10215-901	IL-10	750	0.18	<10	<10
EPXS010-10238-901	IL-12p70	3,090	0.75	<10	<10
EPXS010-12017-901	IL-17A	1,030	0.25	<10	<10
EPXS010-10281-901	MCP-1	650	0.16	<10	<10

\* Upper limit of quantitation. These values are lot specific.

\*\* Lower limit of quantitation. These values are lot specific.

# ProcartaPlex Mix and Match panels

## Design your own multiplex panel



Build the panel of your choice with our online Invitrogen™ ProcartaPlex™ Panel Configurator. Design your own custom assay blend at [thermofisher.com/mixandmatchluminex](https://thermofisher.com/mixandmatchluminex)

ProcartaPlex analytes	Human	Mouse	Rat	NHP	Canine	Porcine
4-1BB (CD137)	•					
4-1BBL (CD137L)	•					
6Ckine (CCL21)	•					
Albumin*			•			
Adiponectin	•	•		•		
Amphiregulin	•					
Amyloid beta (1–40)	•					
Amyloid beta (1–42)	•					
Angiogenin	•					
Angiopoietin-1	•					
Angiostatin	•					
Antithrombin*	•					
Apolipoprotein E4	•					
APRIL	•					
Arginase-1	•					
AXL	•					
B7-H6	•					
BACE1 (beta-secretase 1)	•					
BAFF	•	•				
Beta-2-microglobulin (B2M)	•					
BLC (CXCL13)	•			•		
BDNF	•			•		
Betacellulin (BTC)		•				
Big ET-1	•					
BMP-2	•					
BMP-9	•					
BTLA (CD272)	•	•				
C-peptide	•					
C3a	•					
CA19-9 (Sialyl Lewis A)	•					
CA72-4 (TAG 72)	•					
CA125 (Mucin-16)	•					
Calcitonin	•					
CALR (CRT)	•					
Caspase-3 (total)	•					
Cathepsin D	•					
CD9	•					
CD14	•					
CD27	•	•				

ProcartaPlex analytes	Human	Mouse	Rat	NHP	Canine	Porcine
CD28	•					
CD30	•					
CD30L	•					
CD36	•					
CD40	•					
CD40L	•			•		
CD44	•					
CD44var (6var)	•					
CD63	•					
CD80 (B7-1)	•					
CD81	•					
CD134 (OX40)	•					
CD137L (4-1BBL)		•				
CD276 (B7-H3)	•	•				
CD47 (IAP)	•					
CD48 (BLAST-1)	•					
CD80		•				
CEA (CEACAM-5)	•					
CHI3L1/YKL-40	•					
CKMB	•					
Clusterin*			•			
Clusterin (Apo-J)	•					
CNTF	•					
COMP	•					
Complement factor H	•					
Cortisol	•					
CRP	•	•	•			
CTACK (CCL27)	•					
CTF-1	•					
CTLA-4 (CD152)		•				
Cystin C*			•			
Cystatin C	•					
D-Dimer	•					
Dkk-1	•					
DR6	•					
E-cadherin	•					
EDA-1	•					
EGF	•					
EGFR (ErbB1)	•					

ProcartaPlex analytes	Human	Mouse	Rat	NHP	Canine	Porcine
Elafin	•					
EMMPRIN	•					
ENA-78 (CXCL5)	•	•				
Endoglin (CD105)	•					
Endostatin	•					
Eotaxin (CCL11)	•	•	•	•		
Eotaxin-2 (CCL24)	•					
Eotaxin-3 (CCL26)	•					
EpCAM	•					
E-selectin (CD62E)	•					
Factor V*	•					
Factor VII*	•					
Factor VIII*	•					
Factor IX*	•					
Factor X*	•					
Factor XI*	•					
Factor XII*	•					
Factor XIII*	•					
Fas (APO)	•					
Fas-L	•					
Ferritin	•					
Fetuin-A	•					
FGF-2	•			•		
FGF-21	•					
FGF-23	•					
Fibrinogen	•					
Flt3-ligand	•					
Follistatin	•					
Fractalkine (CX3CL1)	•					
Galectin-3	•					
Galectin-9	•					
GAPDH	•					
GAS6	•					
G-CSF (CSF-3)	•	•	•	•		
GCP-2 (CXCL6)	•					
GDF-15	•					
GDNF	•					
GFAP	•					
GITR (CD357)	•					

ProcartaPlex analytes	Human	Mouse	Rat	NHP	Canine	Porcine
GITRL	•					
Glypican-1 (GPC1)	•					
GM-CSF	•	•	•	•		
gp130 (IL-6RB)	•					
Granzyme A	•					
Granzyme B	•			•		
GRO alpha (KC/CXCL1)	•	•	•			
GLP-1	•					
Ghrelin	•					
Haptoglobin	•					
HB-EGF	•					
HER-2	•					
HGF	•					
HGFR (c-Met)	•					
HMGB1 (HMG-1)	•					
HSP27	•					
HSP60	•					
HSP70	•					
HSP90	•					
HVEM (CD270)	•					
I-309 (CCL1)	•					
IGF-2	•					
IGFBP-2	•					
IGFBP-3	•					
Insulin	•					
ICAM-1	•	•	•	•		
ICOS ligand (B7-H2)	•					
IFN-alpha	•	•		•		•
IFN-beta	•	•				•
IFN-gamma	•	•	•	•	•	•
IFN-omega	•	•				
IgA*	•	•	•			
IgE*	•	•				
IgG1*	•	•	•			
IgG2*	•					
IgG2a*		•	•			
IgG2b*		•	•			
IgG2c*		•	•			
IgG3*	•	•				
IgG4*	•					
IgM*	•	•	•			
IDO	•					
IL-1 alpha	•	•	•			
IL-1 beta	•	•	•	•		•
IL-1 RA	•			•		
IL-2	•	•	•	•	•	
IL-2RA (CD25)	•	•				
IL-3	•	•				

ProcartaPlex analytes	Human	Mouse	Rat	NHP	Canine	Porcine
IL-4	•	•	•	•	•	•
IL-5	•	•	•	•		
IL-6	•	•	•	•	•	•
IL-7	•	•		•		
IL-7RA (CD127)			•			
IL-8 (CXCL8)	•			•	•	•
IL-9	•	•				
IL-10	•	•	•	•	•	•
IL-12/IL-23p40	•	•	•	•	•	•
IL-12 p70	•	•	•	•		
IL-13	•	•	•	•		
IL-15	•			•		
IL-15/IL-15R		•				
IL-16	•					
IL-17A (CTLA-8)	•	•	•	•		
IL-17AF	•					
IL-17F	•	•		•		
IL-18	•	•		•		
IL-19		•				
IL-20	•					
IL-21	•	•	•			
IL-22	•	•				
IL-23	•	•		•		
IL-25		•				
IL-27	•	•				
IL-29 (IFN-lambda 1)	•					
IL-31	•	•				
IL-33	•	•				
IL-34	•					
IL-37	•					
IP-10 (CXCL10)	•	•	•	•		
I-TAC (CXCL11)	•			•		
Kallikrein-6 (KLK6)	•					
KIM-1*			•			
KRT18	•					
Lactoferrin	•					
LAG-3	•	•				
LAP (TGF-beta 1)	•					
LDH-B	•					
Leptin	•	•	•			
LIGHT	•					
LIF	•	•				
LOX-1	•					
Lp-PLA2	•					
L-selectin	•					
LYVE-1	•					
MBL	•					
MCP-1 (CCL2)	•	•	•	•	•	

ProcartaPlex analytes	Human	Mouse	Rat	NHP	Canine	Porcine
MCP-2 (CCL8)	•	•				
MCP-3 (CCL7)	•	•	•	•		
MCP-4 (CCL13)	•					
M-CSF	•	•				
MDC (CCL22)	•					
Mer (MERTK)	•					
Mesothelin	•					
MIA	•					
MICA	•					
MICB	•					
Midkine (MK)	•					
MIF	•			•		
MIG (CXCL9)	•			•		
MIG (CXCL9)		•				
MIP-1 alpha (CCL3)	•	•	•	•		
MIP-1 beta (CCL4)	•	•		•		
MIP-2 alpha (CXCL2)	•	•	•			
MIP-3 alpha (CCL20)	•					
MIP-3 beta (CCL19)	•					
MIP-4	•					
MMP-1	•					
MMP-2	•					
MMP-3	•					
MMP-7	•					
MMP-8	•					
MMP-9	•					
MMP-12	•					
MMP-13	•					
MPIF (CCL23)	•					
Myeloperoxidase (MPO)	•					
Myoglobin	•					
NCAM-1	•					
Nectin-2	•					
Neurogranin (NRGN)	•					
NF-H	•					
NGAL	•		•*			
NGF beta	•		•	•	•	
NRP-1	•					
NSE	•					
NTproBNP	•					
NT5E	•					
Oncostatin M (OSM)	•					
Osteopontin	•		•*			
Osteoprotegerin (OPG)	•					
PAI-1 (Serpin)	•			•		
PDGF-BB	•			•		
PD-1		•				
PD-1 (CD279)	•					

ProcartaPlex analytes	Human	Mouse	Rat	NHP	Canine	Porcine
PD-L1	•					
PD-L1 (CD274)	•					
PD-L2	•	•				
PECAM-1	•					
Perforin	•					
Periostin (OSF-2)	•					
PIGF	•					
Protein C*	•					
Protein S*	•					
Prothrombin*	•					
P-selectin	•					
PTX3	•					
PVR	•					
PYY	•					
RAGE	•					
RBP4	•					
RANKL	•	•	•			
RANTES (CCL5)	•	•	•	•		
REG3a	•					
Resistin (ADSF)	•			•		
S100A8/A9	•					
S100B	•					
SAA	•					
SAP (Pentraxin 2)	•					
SARS-CoV-2 Delta (B.1.617.2)	•					
SCF	•			•	•	
SCGF-beta	•					

ProcartaPlex analytes	Human	Mouse	Rat	NHP	Canine	Porcine
SDF-1 alpha (CXCL12a)	•			•		
Siglec-7	•					
Siglec-9	•					
SPARC (osteonectin)	•					
ST-2	•					
ST2 (IL-33R)	•	•				
Survivin (BIRC5)	•					
Syndecan-1	•					
Syntenin-1	•					
TARC (CCL17)	•					
Tactile	•					
Tau (Total)	•					
Tau [pT181]	•					
Tau [pT231]	•					
TDP-43	•					
TECK (CCL25)	•					
Tenascin-C	•					
TFF-3*			•			
TGF-alpha	•			•		
TGF-beta 1	•	•	•	•	•	•
Thrombopoietin (TPO)	•					
TIE-2	•					
TIM-3 (CD366)	•	•				
TIMD-4	•					
TIMP-1	•		•*			
TNF-alpha	•	•	•	•	•	•
TNF-beta	•			•		
TNF-R1	•					

ProcartaPlex analytes	Human	Mouse	Rat	NHP	Canine	Porcine
TNF-R2	•					
TNI	•					
tPA	•					
TRAIL	•			•		
TRAIL-R1	•					
TRAIL-R2	•					
TREM-1	•					
TREM-2	•					
TrkB	•					
TSLP	•	•				
Tweak	•					
TYRO3	•					
UCHL1	•					
ULBP-1	•					
ULBP-3	•					
ULBP-4	•					
uPAR	•					
VCAM-1	•		•	•		
VE-cadherin	•					
VEGF-A	•	•	•	•	•	
VEGF-D	•			•		
VEGF-R1	•					
VEGF-R2	•					
VEGF-R3	•					
VISTA (B7-H5)	•					
VLA-4	•					
vWF*	•					
ZAG	•					

\* Analytes only available as part of a panel.

## Sample measurement service

No Luminex instrument available? Sending us your samples to be measured on a ProcartaPlex panel can help save you time and money. Our service team consists of highly skilled and experienced professionals who will work with you throughout the stages of your project to help ensure delivery of high-quality data and results.

### Benefits of our custom assay service:

- Enables you to obtain reproducible, quantitative data from a small volume
- Receive high-quality assay and data reports created by experienced professionals
- Eliminate the need to buy a Luminex instrument
- Free up time and resources to focus on other projects

# Multiplex biomarker quantitation of mRNA targets



## QuantiGene Plex multiplex assays

Accurate and precise RNA quantitation of up to 80 gene transcripts in a single well (96- and 384-well format)

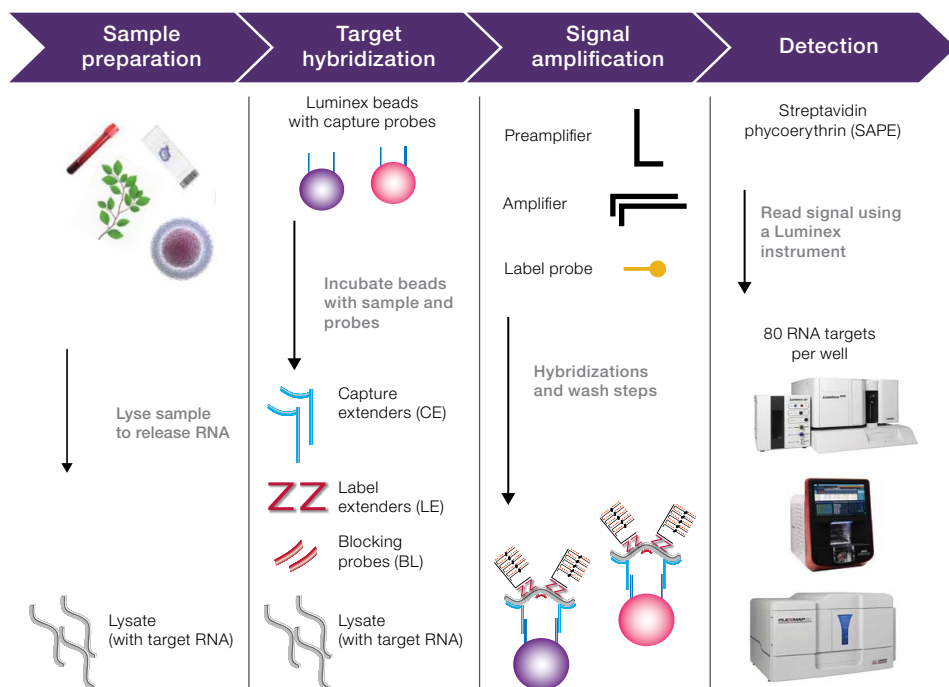
### Overview of QuantiGene Plex assays

The QuantiGene Plex assays help provide an accurate and precise method for multiplexed gene expression quantitation. QuantiGene Plex assays utilize the same Luminex xMAP technology you're familiar with for multiplex protein quantitation. With QuantiGene assays, instead of measuring protein, you're able to simultaneously measure 3–80 RNA targets in every well of a 96- or 384-well plate. Branched DNA assays allow for the direct measurement of RNA transcripts by using signal amplification rather than template amplification.

The QuantiGene Plex assays are simple and easy to use; they don't require RNA extraction, cDNA synthesis, or PCR amplification. QuantiGene Plex assays are ideal for verifying microarray or next-generation sequencing (NGS) data, and for discovering and verifying biomarkers for translational research.

### Key features and benefits of the assay

- **Works with difficult sample types**—works with degraded and crosslinked RNA in FFPE tissues and directly with blood, with no RNA purification required
- **True multiplexing**—measure up to 80 genes of interest and housekeeping genes in the same well with no cross-reactivity; reduces the number of sample wells and sample amount needed
- **Standardized platform**—compatible with all Luminex instrument systems, allowing you to explore both proteomics and genomics on the same platform
- **Simple workflow**—ELISA-like workflow for direct hybridization of transcripts to beads and transcript labeling, and no reverse transcription needed
- **Large inventory of verified genes**—over 22,500 genes can be mixed in up to 80-plex panels to create pathway- and disease-themed panels
- **Faster customization than other methods**—if we don't have your gene(s), we can create and perform QC on your custom panel typically in 3–4 weeks



Learn more at [thermofisher.com/quantigene](https://thermofisher.com/quantigene)



## Analyze both protein and gene expression from a single sample

Protein and gene expression data can be generated from the same sample, giving researchers more information about that sample (Figures 18 and 19). For example, after a cell culture is centrifuged, the supernatant is analyzed for multiple cytokines using the ProcartaPlex assay. The cell pellet is then lysed and analyzed using the QuantiGene Plex assay to obtain gene expression data.

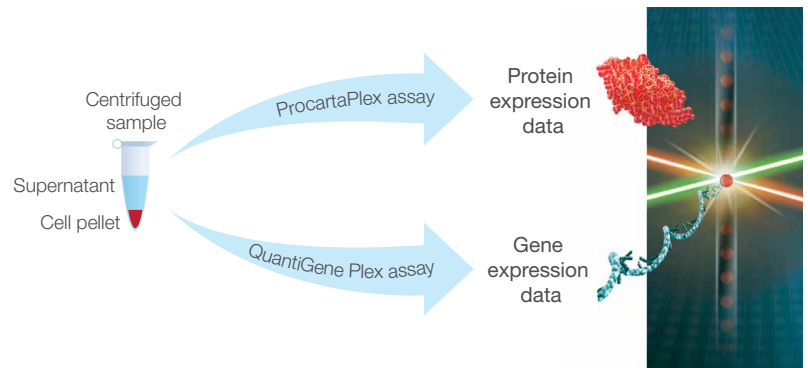


Figure 18. Protein and gene expression data from a single sample.

## Multiplex application—analysis of protein and gene expression from the same sample

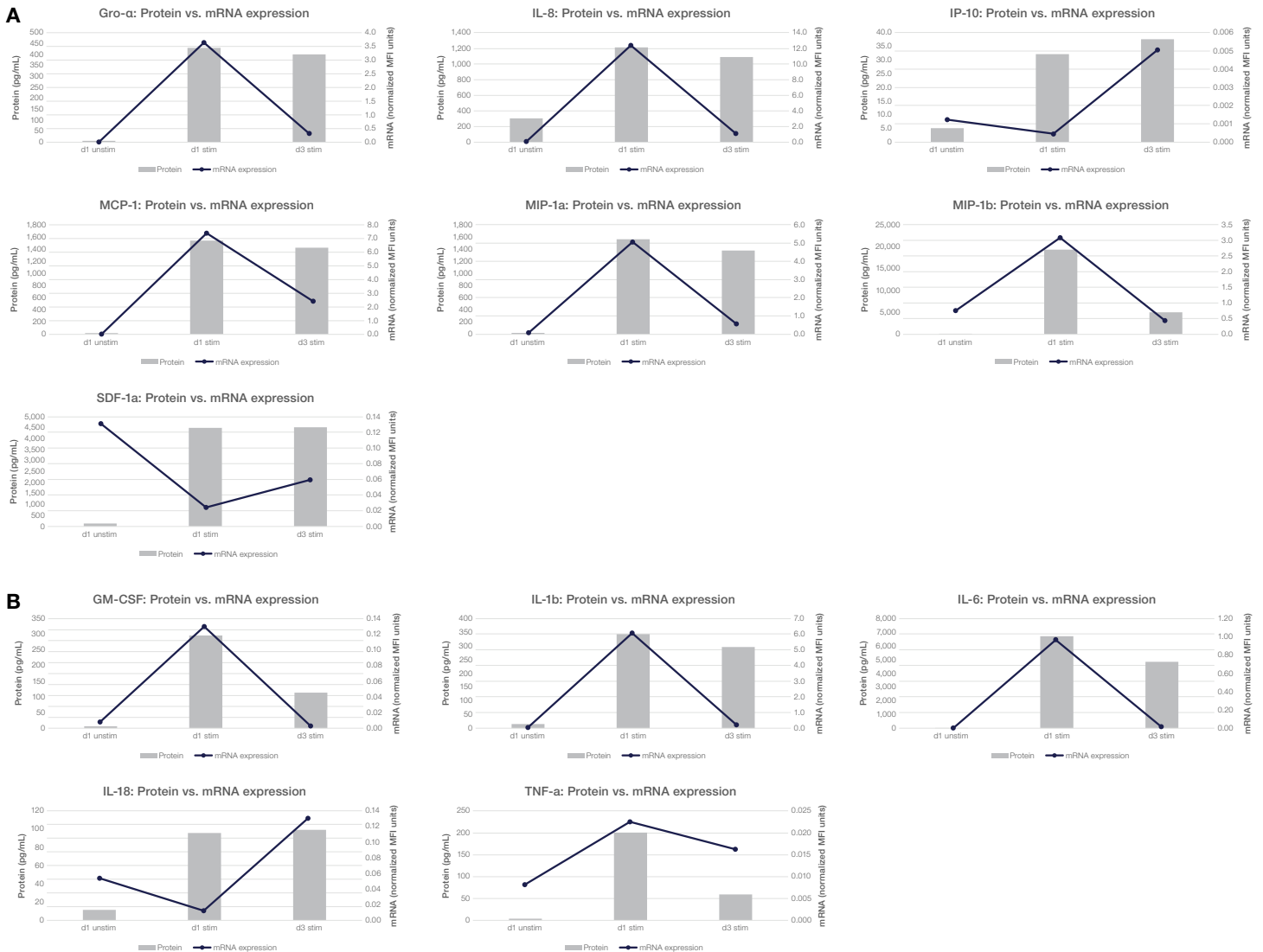
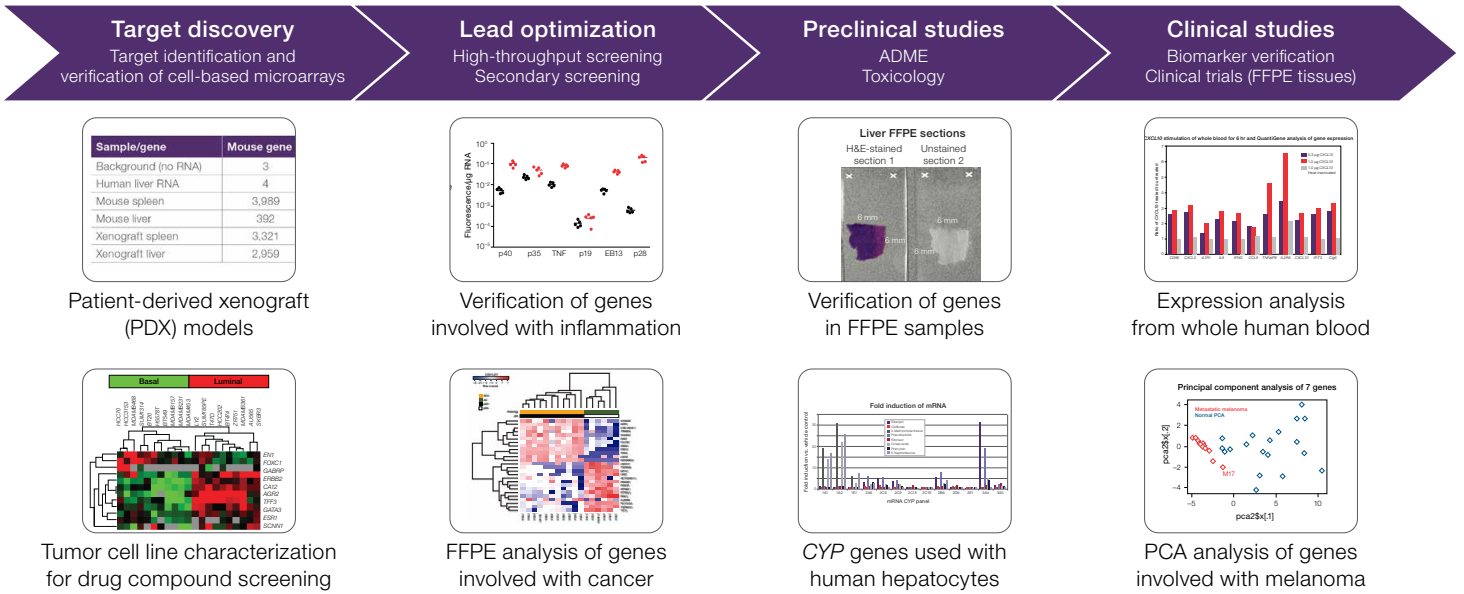


Figure 19. Protein and gene expression analysis of several cytokines. Human PBMCs were isolated using Ficoll-Paque™ PLUS medium (Cytiva, Cat. No. 17-1440-02) and grown in Gibco™ CTS™ OpTmizer™ T Cell Expansion SFM (Cat. No. A10221-01). The cells were then stimulated with 10 µg/mL LPS for up to 72 hours. Cells were lysed using the Invitrogen™ QuantiGene™ Sample Processing Kit for cultured cells (Cat. No. QS0100) at 24 (d1) and 72 hours (d3), and supernatants were tested for cytokine, chemokine, and growth factor proteins in the Invitrogen™ ProcartaPlex™ Human 65-plex panel (Cat. No. EPX650-10065-901) and in an identical biomarker configuration for mRNA using the Invitrogen™ QuantiGene™ Plex Assay Kit (Cat. No. QP1013). The data show a sampling of the results for (A) inflammatory chemokines and (B) Th1/Th2 cytokines, and demonstrate the ease of multiplex measurement of proteins and RNA from a single sample. MFI = median fluorescence intensity. Additional data can be found at [thermofisher.com/quantigene](https://www.thermofisher.com/quantigene).

## QuantiGene Plex assay applications

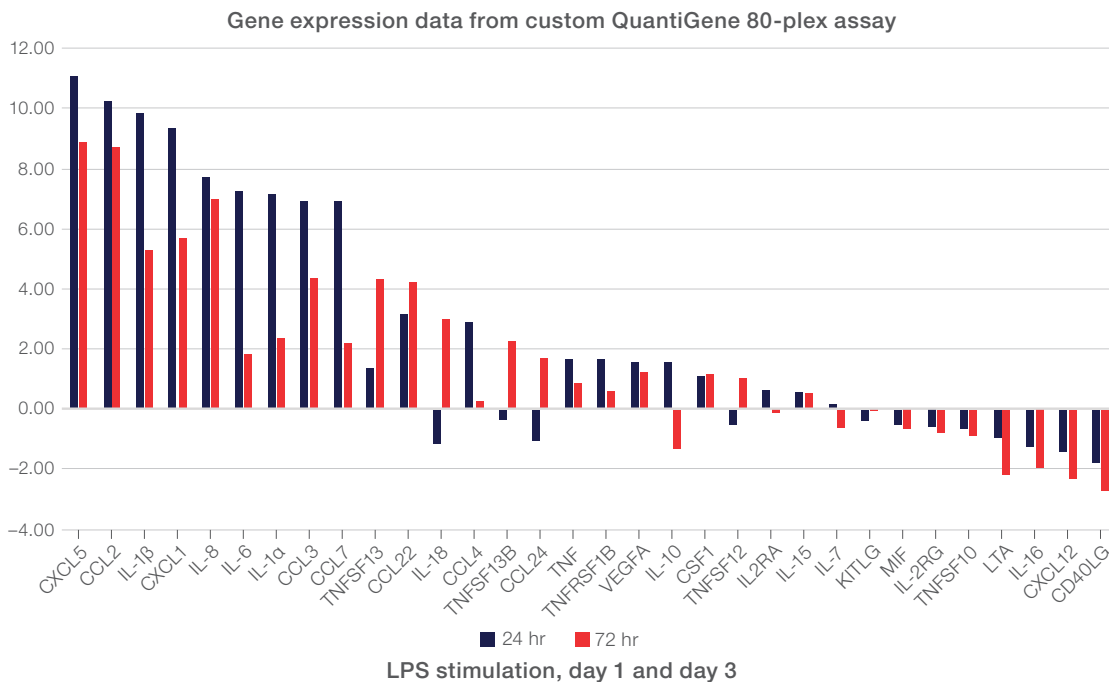
QuantiGene Plex assays for RNA profiling are ideal for supporting drug discovery and development efforts, as well as translational and clinical research. The examples below highlight the capabilities and benefits of this powerful research tool.



## Gene expression data from custom QuantiGene 80-plex assay

LPS stimulation of PBMCs is known to induce cytokine production, including interleukin-1 $\beta$  (IL-1 $\beta$ ), interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). To test the fidelity of QuantiGene assays in measuring changes in mRNA expression levels, a custom QuantiGene 80-plex panel was developed that included 68 mRNA targets and 12 additional reference genes for data normalization. Figure 20 shows select mRNA expression changes in the LPS-stimulated PBMC lysate probed with the QuantiGene 80-plex panel. Increased mRNA fold changes were seen in IL-1 $\beta$ , IL-6, TNF- $\alpha$ , CCL4, and CXCL5 by day 1 and day 3 stimulation over unstimulated day 1.

Other large changes included monocyte chemoattractant protein 1/CCL2 (MCP-1), chemokine (C-X-C motif) ligand 1 (CXCL1), interleukin-8 (IL-8), interleukin-1 $\alpha$  (IL-1 $\alpha$ ), macrophage inflammatory protein-1 $\alpha$ /CCL3 (MIP-1 $\alpha$ ), and monocyte-chemotactic protein 3/CCL7 (MCP3), which all showed a greater than 2-fold expression level increase.



**Figure 20. Thirty-two gene targets showed expression changes with LPS stimulation.** Raw MFI data from the QuantiGene Plex assay were normalized to the geometric mean of the six most stable reference genes according to the geNorm algorithm (out of 12 reference genes in the panel).

## QuantiGene Plex sample measurement services

Accelerate your research by letting us test and analyze your samples using QuantiGene assays, freeing up your valuable resources for other discovery research. Our assay service is designed to help you meet your specific project goals and timelines.

### Our QuantiGene Plex sample measurement services offer the following features and benefits:

- **Consistency**—all assays and reagents used in the service are from Thermo Fisher Scientific
- **Design support**—help save time by accessing our bioinformatics team to customize your probe and panel design
- **Flexibility**—sample types include purified RNA, PAXgene blood, cell lysates and tissue homogenates
- **Choice**—a comprehensive list of applications



Find out more at [thermofisher.com/quantigeneassayservice](https://thermofisher.com/quantigeneassayservice)

## QuantiGene Plex data analysis software

A cloud-based software is available to support data analysis for QuantiGene Plex gene expression assay results. The software includes an intuitive workflow that guides you step by step once you have uploaded your run data from your Luminex instrument. Easily assign control and treatment groups, select QC parameters, customize data QC, and perform data normalization. Access the cloud-based software at [apps.thermofisher.com/apps/quantigene](https://apps.thermofisher.com/apps/quantigene)

Analyzed data can be exported to the Applied Biosystems™ Transcriptome Analysis Console (TAC) software for advanced analysis and visualization. When combined with TAC, the analysis allows for better visualization and interpretation using representations like scatter and volcano plots, hierarchical clustering, and links to publicly available annotations. Download a free copy of TAC software at [thermofisher.com/tacsoftware](https://thermofisher.com/tacsoftware).

### Available features:

- Analyze data from 96- and 384-well plates
- Rank normalization genes and calculate *P* values during fold-change analysis
- Group samples and analyze fold change in groups
- Export data for advanced analysis using TAC software

### Specifications

The QuantiGene Plex assay can be used for RNA profiling.

Feature	Specification
Limit of detection	≤1,000–2,000 transcripts/assay well
Limit of quantitation	≤2,000–4,000 transcripts/assay well
Linear dynamic range	≥3.0 logarithmic units
Assay CV	≤15% intra-assay; ≤20% inter-assay
Compatible sample types	Cultured cells, bacteria, whole blood, PAXgene™ samples, Invitrogen™ Tempus™ samples, dried blood spots, fresh-frozen tissues (animal or plant), FFPE samples, purified RNA
Assay format	96- or 384-well plate
Targets/well	3–80

# Ordering options for QuantiGene Plex panels

## Off-the-shelf panels

We have developed more than 5,200 panels that include more than 22,500 genes. These available panels cover a wide range of host species that include human, mouse, rat, canine, monkey, porcine, soy, maize, and many others. Any combination of existing genes can be combined to create a new panel. To find the latest panels and genes, go to [thermofisher.com/qgppanels](https://thermofisher.com/qgppanels) or contact one of our probe designers at [lsq.qgprobe@thermofisher.com](mailto:lsq.qgprobe@thermofisher.com).

## QuantiGene Plex panel configurator for custom panels

Custom QuantiGene Plex panels are available for any gene and any species. Custom panels ranging from 3- to 80-plex can be designed and shipped typically within 2–3 weeks. Customers simply provide us with a gene list, RefSeq IDs, or DNA sequences (Figure 21).



Visit [thermofisher.com/qgp-panelconfigurator](https://thermofisher.com/qgp-panelconfigurator)

The screenshot displays the ThermoFisher Scientific website's QuantiGene Plex panel configurator. The main heading is "Select genes". Below it, there's a search bar and filters for "Filter by species" (set to Human) and "Filter by category". A table lists various gene categories with "view genes" links and associated publications. On the right, a "Summary" section shows a "Targets 14 / 80" list with columns for "Gene Symbol" and "Species".

Gene Symbol	Species
1. Ase1	Rt
2. Btk	Po
3. CASP2	Ck
4. KLLN	Zf
5. LOC100194714	Cp
6. LOC100362113	Rt
7. LOC100423217	Gr
8. LOC100498592	Gr
9. M3M4	Cp
10. pser	Fa
11. PDRG1	Ov
12. PERP	Ma
13. p95	Cp
14. PRG1	Rt

Figure 21. QuantiGene Plex panel configurator tool.

# Luminex instrumentation

The Luminex family of instruments includes options for various multiplexing capabilities, throughputs, and read times. All instruments are capable of protein and nucleic acid applications, and all are supported by commercially available assays that can multiplex up to 80 biomarkers simultaneously. That's why Luminex instruments, paired with ProcartaPlex and QuantiGene assays, create your all-in-one platform for studying both proteomics and genomics. Compare the systems below to determine which Luminex instrument is right for your research.



Instrument	xMAP INTELLIFLEX DR-SE	xMAP INTELLIFLEX	FLEXMAP 3D	Luminex 200
	Most advanced Luminex platforms		Higher-throughput multiplexing	Most established platform
Applications	Protein and nucleic acid			
Multiplex capacity	Up to 500			Up to 100 (80 on MagPlex microspheres)
Max panel size	80 biomarkers			
Sensitivity	0.06–1 pg/mL (immunoassays; assay and target dependent) 1,000 RNA copies per target/well (RNA assays)			
Dynamic range	≥5.5 logarithmic units (RP1) ≥4.5 logarithmic units (RP2)	≥5.5 logarithmic units (RP1)	≥4.5 logarithmic units (RP1)	≥3.5 logarithmic units (RP1)
Sample volume (assay dependent)	6.3–80 µL			25–80 µL
Plate format	96-well 384-well			96-well
Read time	20 min (96-well) 75 min (384-well)			45 min (96-well)
Footprint	58.4 x 61 x 76 cm		110 x 62 x 63 cm	115 x 60 x 50 cm
Optics/hardware	Flow cytometry-based lasers, APDs, PMTs			
Reporter laser/optics	532 nm (green) and 405 nm (violet)	532 nm (green)	532 nm (green)	532 nm (green)
Operational validation/compliance	Calibration, verification, and fluidics 21 CFR compliance (coming in 2023 for INTELLIFLEX instrument)			
Automation	Automation integration software available (coming in 2023 for INTELLIFLEX instrument)			
Analytics (software)	Data analysis apps for Luminex assays on the Connect Platform		xPONENT® basic plus partner analytics	

Accessories to keep your Luminex instruments running well include sheath fluid, calibrator and control microspheres, verification and calibration kits, and hand-held plate washers.



Learn more at [thermofisher.com/luminexinstruments](https://www.thermofisher.com/luminexinstruments)

# Additional solutions for gene expression analysis

## Gene expression profiling solutions

QuantiGene assays coupled with Luminex instrumentation or plate readers provide a unique and validated option for multi-omics work. Additional comprehensive solutions for gene expression profiling, verification, and analysis are supported by sequencing, microarrays, and real-time PCR technologies. For discovery, in particular *de novo* discovery of differentially expressed genes (DEGs), transcriptome sequencing provides a hypothesis-neutral approach that allows interrogation of any gene in the sample, with a wide dynamic range to allow differential gene expression analysis of low-expressing genes. For analysis deep within the known transcriptome, transcriptome-level microarrays provide fast profiling of coding and noncoding genes, exons, and transcript isoforms. Well-annotated gene expression analysis can be performed with gene-level microarrays. DEGs can be verified using mid-throughput real-time PCR formats such as Applied Biosystems™ TaqMan™ OpenArray™ Panels and TaqMan™ Array Cards, and the resulting genes of interest can be quantified more precisely by digital PCR or real-time PCR with Applied Biosystems™ TaqMan™ Gene Expression Assays. Testing experiments can also be performed to further examine the behavior of these genes across a larger population of samples.

## Gene expression profiling by sequencing

You can get the same simple workflow with the same flexibility, now faster and more scalable than ever. The Ion Torrent™ GeneStudio™ S5 series is a line of benchtop NGS systems that enable you to efficiently run small and large projects across multiple research applications, with the simplest sample-to-data benchtop NGS workflow and superior speed. With flexibility to choose from five Ion Torrent™ chips, these systems offer an opportunity to conduct wide-ranging experiments on a single platform. A single instrument can scale easily to accommodate multiple RNA analysis applications, throughputs, and data outputs including:



- Whole-transcriptome analysis
- Small RNA analysis
- Targeted RNA sequencing

Find out more at [thermofisher.com/genestudio](https://thermofisher.com/genestudio)

## Gene expression profiling by microarrays

Accelerate your expression biomarker discovery research with Applied Biosystems™ Clariom™ assays, the next generation of transcriptome profiling tools offering a fast path to results.



- Generate expression profiles from as little as 100 pg of total RNA—as few as 10 cells
- Analyze RNA from a wide variety of sample types, including cells, whole blood, and fresh or fresh-frozen, or FFPE tissues
- Preserve sample integrity and reduce variability with no need for globin or ribosomal RNA removal
- Transcriptome profiling of coding and long noncoding genes, exons, and splice variants with the Applied Biosystems™ Clariom™ D Pico Assay
- Well-annotated gene-level expression profiling with the Applied Biosystems™ Clariom™ S Pico Assay
- Short noncoding RNA profiling with the Applied Biosystems™ GeneChip™ miRNA 4.0 Assay

Find out more at [thermofisher.com/transcriptomeprofiling](https://thermofisher.com/transcriptomeprofiling)



### Gene expression analysis by real-time PCR

Real-time PCR, or qPCR, is the preeminent technique for verification of differential gene expression profiles. Our comprehensive offering of TaqMan Gene Expression Assays and flexible formats enable fast and cost-effective results. We are the leader in gene expression analysis, providing world-class sample preparation with Applied Biosystems™ technologies, and real-time PCR using TaqMan or Applied Biosystems™ SYBR™ Green chemistry, the popular Applied Biosystems™ QuantStudio™ portfolio of real-time PCR instruments, as well as data analysis software.

Applied Biosystems™ TaqMan™ Assay technology is the gold standard in performance, quality, and content for gene expression analysis. With predesigned TaqMan Assays, spend your time generating results, not designing and optimizing assays.

- Detect virtually any gene product—more than 2.8 million predesigned assays, and custom designs for everything else
- Assays for nearly every human, mouse, and rat gene in the RefSeq database
- Available for 32 species, and some pathogens
- Assays for multiple locations per transcript and across nearly every exon junction in human genes
- Predesigned TaqMan Assays are backed by a performance guarantee\*

Find out more at [thermofisher.com/qpcr](https://thermofisher.com/qpcr)

\* Terms and Conditions apply. For full details, go to [thermofisher.com/taqmanguarantee](https://thermofisher.com/taqmanguarantee)



### Gene expression quantification by digital PCR

Digital PCR enables highly precise measurements and is able to resolve changes in gene expression level of two-fold or less. Digital PCR can also be used to determine the absolute quantification of a transcript without the need for a reference gene.

Now, you can go beyond the limits of real-time PCR with the Applied Biosystems™ QuantStudio™ 6 Flex Real-Time PCR System. The system is ideal for laboratories with multiple applications or end users on a limited budget. Easily interchangeable thermal cycling block formats let you select the format (96-well, 96-well Fast, or 384-well) that best suits your project without having to move attached peripherals or computers. The intuitive software, easy touchscreen setup, and effortless block changes are designed to let you get started right away.

Find out more at [thermofisher.com/quantstudio6flex](https://thermofisher.com/quantstudio6flex)

# High-throughput quantitation

## ProcartaPlex and QuantiGene Plex 384-well assays

ProcartaPlex and QuantiGene Plex assays help enable a unique high-throughput multi-omics approach utilizing the Luminex platform. Proteomic and genomic workflows can be combined without compromising data interpretation or sensitivity.

Investigate cell functions and responses by simultaneously interrogating large sets of RNA or protein targets in single samples in either 96-well or 384-well format (Table 5).

Table 5. High-throughput protein and gene expression assays.



	ProcartaPlex assays	QuantiGene Plex Assay
Intra-assay CV	<15%	
Inter-assay CV	<15%	
Linearity	3–5 logarithmic units	
Maximum assay plex size	65 proteins	80 RNA targets
Formats	96- and 384-well	
Sample types	Serum, plasma, cell culture supernatant (CCS), cerebrospinal fluid (CSF)	RNA; cell and blood lysates; tissue and FFPE homogenates
Species	Human, mouse, rat, canine, porcine, nonhuman primate	All
Compatible Luminex instruments	xMAP INTELLIFLEX System FLEXMAP 3D System Luminex 200 System MAGPIX System	
Sample volume	6.3–50 µL	20–80 µL

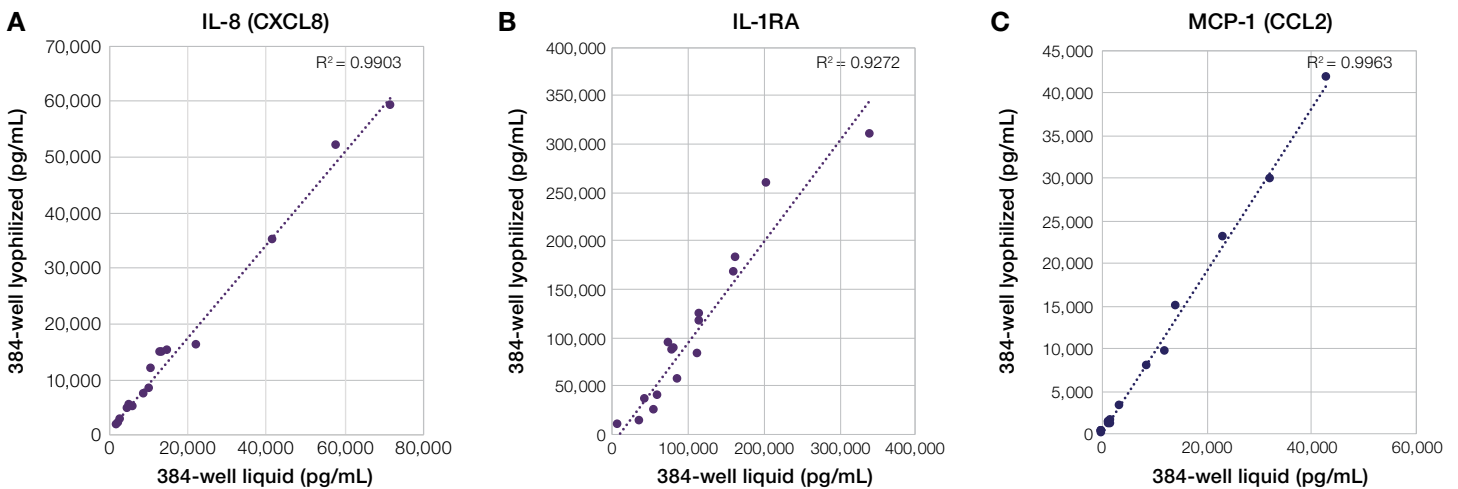


## Protein multiplex assays

ProcartaPlex immunoassays are antibody-based, magnetic bead reagent kits and panels for multiplex protein quantitation on the Luminex instrument platform. We provide the flexibility to mix and match your own panels with a menu that includes more than 500 cytokines, chemokines, growth factors, and other protein targets from a range of species. The newest ProcartaPlex product line includes lyophilized bead kit formats for an easier workflow that helps to reduce hands-on time, enabling faster results.

### Features include:

- **Higher throughput without compromising sensitivity**—increase throughput and sensitivity with only 6.3–50  $\mu\text{L}$  of sample required
- **Easily create your own panels**—use premixed custom combinations from our broad list of targets
- **Consistent results**—scalable and reproducible performance regardless of multiplex level
- **Lyophilized assay format available**—requires less hands-on time for faster results with identical performance as liquid bead format (Figure 22)



**Figure 22. Identical performance of ProcartaPlex 384-well assays in lyophilized and liquid bead formats.** ProcartaPlex 384-well assays are available in two formats: ready-to-use plates with pre-dispensed lyophilized beads for workflow convenience (384-well lyophilized) and a liquid formulation of bead sets in a vial to be pipetted onto a 384-well flat bottom plate (384-well liquid). Data of selected analytes in the graphs [(A) IL-8 (CXCL8), (B) IL-1RA, (C) MCP-1 (CCL2)] illustrate sample correlations between the 384-well lyophilized and 384-well liquid formats obtained for 16 human plasma samples. The high sample coefficients ( $R^2$ ) clearly demonstrate identical performance of ProcartaPlex 384-well assays with lyophilized and liquid bead formulation.

“Our core facility has been using the Invitrogen™ Th1/Th2 Cytokine 36-Plex Mouse ProcartaPlex™ Panel on tissue lysates. We have many customers who have been pleasantly surprised by the reproducibility of the assay and some have commented: ‘This assay nicely validated what we have found in the gene expression.’”

—Diane Bender, PhD

The Bursky Center for Human Immunology and Immunotherapy Programs  
Washington University School of Medicine

Learn more at [thermofisher.com/procartaplex](https://thermofisher.com/procartaplex)

## RNA multiplex assays

QuantiGene Plex assays are solutions for fast, high-throughput multiplex quantitation of gene expression and allow for the simultaneous measurement of up to 80 genes in one well of a 96- or 384-well plate. QuantiGene Plex assays are hybridization-based and incorporate branched DNA (bDNA) technology, which uses signal amplification rather than target amplification for direct measurement of RNA transcripts. The assays are extremely easy to run using a simple ELISA-like workflow that does not require RNA purification.

### Features include:

- **No RNA purification step needed**—simply lyse and go
- **Compatible with difficult samples**—including blood and degraded RNA in FFPE tissues
- **Design your own panels**—our expert bioinformatics team will design probes for any target or species
- **Easy batch processing**—for higher throughput (Figure 23)

### A

Parameter	First plate	Last plate
Signal relative to first plate (%)	100%	115%
CV	18%	18%
Linearity	111%	106%

### B

Hybridization step	30 min hold before hybridization	30 min hold after hybridization
Pre-Amp	102%	104%
Amp	103%	107%
Label probe	105%	105%
Streptavidin-PE	111%	103%

**Figure 23. Consistency in performance between first and last plate of a 10-plate batch enables higher throughput.** The new workflow has an assay tolerance of 30 minutes and is verified for processing 10 plates with automation equipment. The time lag between plates in the workflow does not affect the performance of individual hybridization steps. The normalized signals remain robust and consistent. **(A)** Comparison of assay specifications comparing first and last plate of the batch. The time lag does not significantly affect assay performance of the hybridization steps investigated. All plates have CVs and linearity within 20%. The variation in normalized signal is <15%. **(B)** Comparison of signals for plates held at different steps of the assay for 30 min before and after the particular step.

### Build the panel you need

Design a customized and verified QuantiGene Plex assay at [thermofisher.com/order-quantigene-plex](https://thermofisher.com/order-quantigene-plex)

Learn more at [thermofisher.com/quantigene](https://thermofisher.com/quantigene)

“The versatility and sensitivity of the QuantiGene Plex assay using xMAP bead-based technology enables multiplex RNA quantification readouts, offering opportunities for cancer biomarker validation studies in retrospective material and robust measurements in liquid biopsies. This technology is instrumental in our success to develop novel biomarker panels in various scientific fields.”

—Professor Godfrey Grech, PhD  
Department of Pathology  
Faculty of Medicine and Surgery  
University of Malta

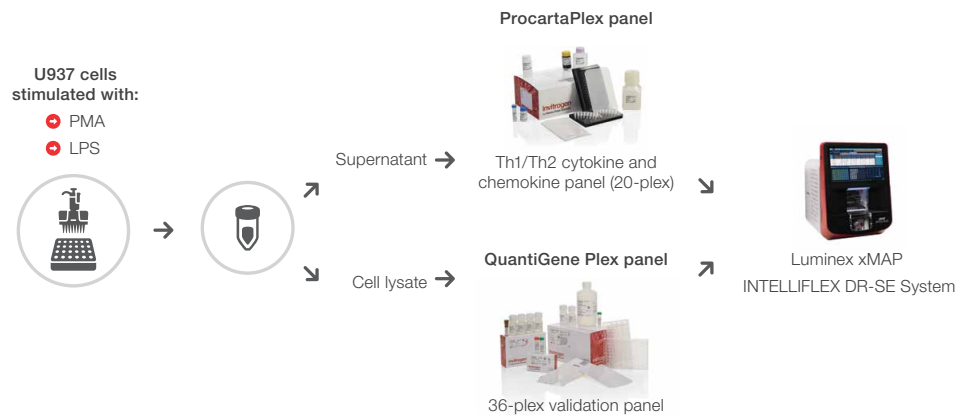
## Two assays, one technology

### High-throughput measurements of protein and gene expression on a single platform

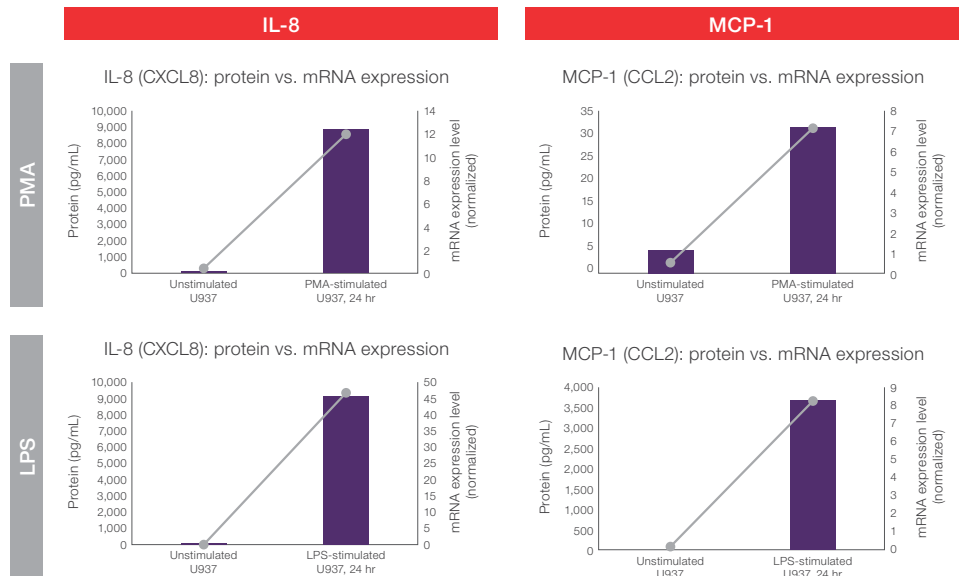
The ability to perform both RNA and protein measurements using unified, high-throughput workflows makes the ProcartaPlex and QuantiGene Plex assays ideal tools for large-scale screening studies that benefit from automated workflows. A more robust and accurate picture of cell states can be obtained by examining variations in both RNA and protein expression. RNA levels can be transient and may not correlate with protein levels, which are likely to remain stable for longer periods of time to maintain cellular functions. Due to differences between timing and expression levels, one assay may detect changes that the other does not. Therefore, analyzing both data sets can provide more detailed insights.

### Workflow description

In a typical workflow, cells are treated with various stimulants or compounds. Cell lysates are collected to analyze RNA, membrane proteins, and intracellular proteins, while cell supernatants are collected to analyze cytokines or other secreted proteins. Samples are then interrogated with kits that target the analytes of interest (Figures 24 and 25).



**Figure 24. Up to 65 protein and 80 RNA targets can be measured in a single sample in a 384-well format.** U937 cells cultured in 384-well plates were stimulated with PMA (24 hours) or LPS (48 hours). The cell culture supernatants were collected, and secreted proteins were measured using the Invitrogen™ ProcartaPlex™ Mouse Th1/Th2 Cytokine Panel 20-Plex. The cells were lysed using Invitrogen™ QuantiGene™ Lysis Mixture, and changes in gene expression were quantified using an Invitrogen™ QuantiGene™ Plex 36-plex panel. Both assays were run on a Luminex xMAP INTELLIFLEX DR-SE System.



**Figure 25. Increase throughput without compromising sensitivity.** Total protein quantitation (bar graphs) and normalized gene expression analysis (line graphs) show an excellent correlation between gene expression and protein translation for the representative targets MCP-1 (CCL2) and IL-8 (CXCL8) after cells were stimulated with PMA or LPS.



### Publication:

Multiplexing protein and gene level measurements on a single Luminex platform

To read the full publication, visit [thermofisher.com/luminex](http://thermofisher.com/luminex)

# Laboratory automation solutions

## Innovative mechanical integrity for secure automation

Multiplex protein and gene expression assays can be automated with the help and support of our laboratory automation team, bringing to your lab decades of experience in automated incubation, laboratory robotics, and workflow scheduling. Our dedicated team of specialists provides automation support and experience to meet your laboratory's high-throughput needs.

We provide scalable solutions for single-step liquid transfers all the way up to full systems that require reagent dispensing, plate sealing and peeling, and magnetic bead washing. Solutions for multiple throughputs with partial plates or multiple plates in the same run as well as customizable setups and concepts for ProcartaPlex and QuantiGene Plex assays are available.



## Automation—not just for throughput

Automation can improve more than just capacity and throughput.



### Decreases hands-on time

Increase walk-away time to maximize skilled labor resources



### Maximizes throughput

Boost instrument uptime and number of runs



### Reduces error

Reduce the number of manual processes and opportunities for human error



### Generates reproducible results

Improve sample processing uniformity



### Enables flexible scaling

Adapt to current workflows and future capacity needs



### Supports social distancing

Work remotely with the help of automation

Learn more about laboratory automation solutions at [thermofisher.com/labautomation](https://www.thermofisher.com/labautomation)

# Immunoassay special services

Our immunoassay R&D team has over 30 years of experience with immunoassay development, sample handling, data analysis, and project management.

Operating in an ISO 9001–certified facility, we employ high-quality standards and procedures.

- Not sure where to start? We offer valuable consultation services, free of charge.
- Can't find a commercially available assay for your target? We offer custom assay development.
- Short on staff resources? Tight deadlines? Or just want experienced scientists to run your samples and provide results to you? We offer sample testing services.
- Starting a large or long-term research project? We offer helpful services like special bulk packaging and lot reservation.

## Custom immunoassay development and services

- Have us develop a custom assay unique to your target, with project milestones and progress reports
- Request custom plate coating to enable consistency across experiments (ELISA only)
- Request additional QC testing of assays

## Special packaging

- Obtain products in bulk packaging for less waste, better use of storage space, or high-throughput automation needs
- Request additional components or special formulations

## Sample testing services

- Send us your samples and we will run the assay for you

## Lot reservation

- Request multiple kits from a single lot for large or long-term studies

## Immunoassay support center

### Assay development service

Custom assay development services are available for protein targets that are not commercially available.

By using our services, you leverage years of assay development experience without wasting your time. Let us help save you time so you can work on the important parts of your research.

- Utilize technical specialists with over 30 years of assay development experience
- Save time and money, and avoid the hassle of optimizing and troubleshooting
- Be confident that you're getting the best assay possible

### Sample testing service

No Luminex instrument available? Not sure if this is the right platform for you? Our service team comprises highly skilled and experienced professionals who will work with you throughout the stages of your project to help ensure delivery of high-quality data and results.

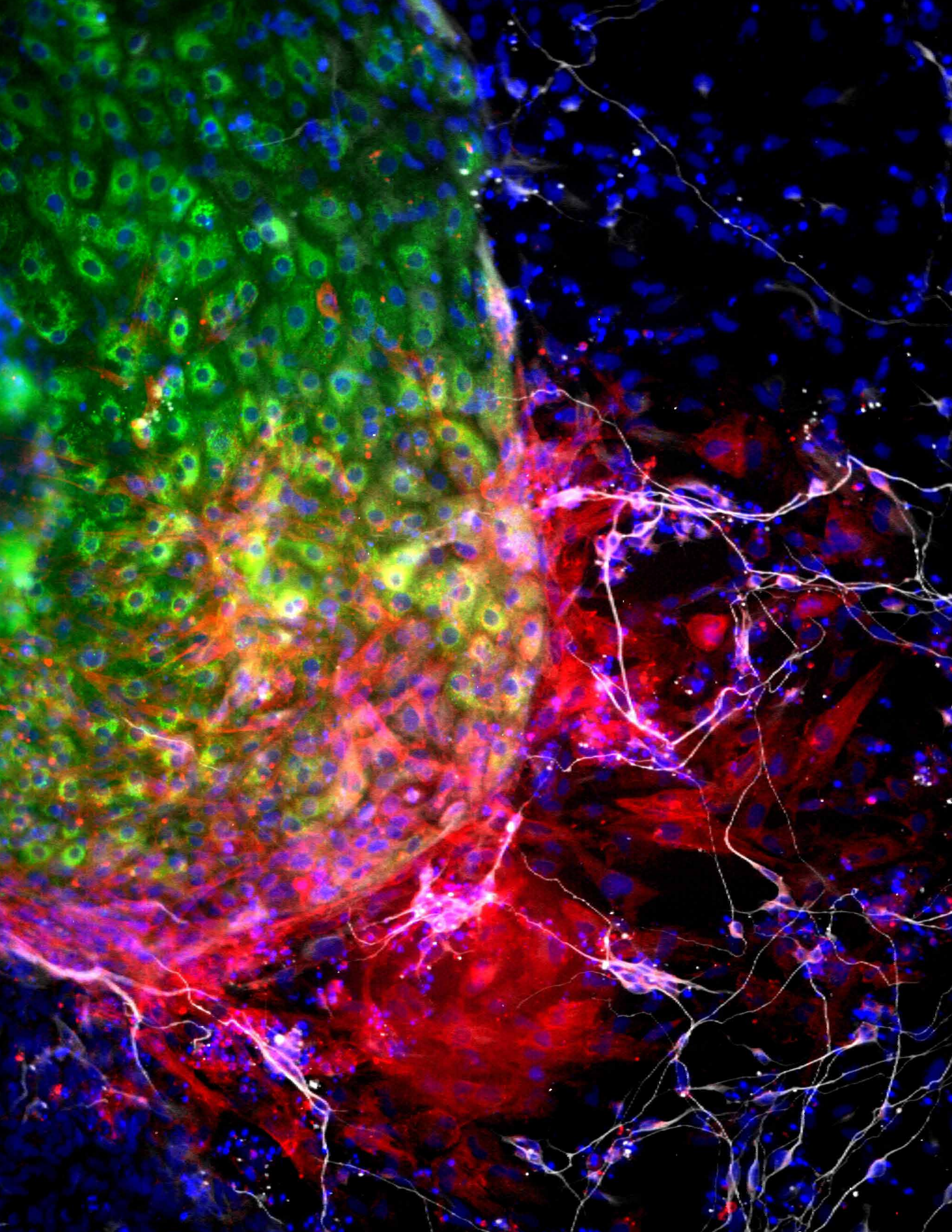
### This allows you to test the technology and see if the data meets your expectations.

- Be confident that your samples are being tested by experienced technical specialists
- Receive high-quality reports of your assay data
- Minimal investment needed to make a decision before investing in the Luminex platform



[Access the custom assay services](#) quote request form and find out more about sample testing services at [thermofisher.com/iaspecialservices](https://www.thermofisher.com/iaspecialservices)





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