ThermoFisher SCIENTIFIC

Luminex instrument and assay guide

High-throughput multiplexing of protein and gene expression analysis

invitrogen

The power of multiplexing

Assays and instruments for protein and RNA targets

What is multiplexing?

Multiplexing refers to a technique that allows simultaneous detection of multiple analytes within a single sample. Luminex® xMAP® (multi-analyte profiling) technology combines advanced fluidics, optics, and fluorescently dyed microspheres to help researchers get more data from their precious sample and obtain a comprehensive view of the sample profile.

Advantages of multiplexing

Cost and time savings—measure up to 80 analytes in a single well

Less sample—6.3–50 µL of sample is needed

More answers—get up to 30,720 data points in one plate

Exceptional accuracy—reliably detect proteins and genes across a broad concentration range

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. Invitrogen™ ProcartaPlex™ and Invitrogen™ QuantiGene™ Plex assays are available for analysis of up to 80 analytes in 96-well and 384-well formats for high-throughput analysis.

ProcartaPlex multiplex immunoassays

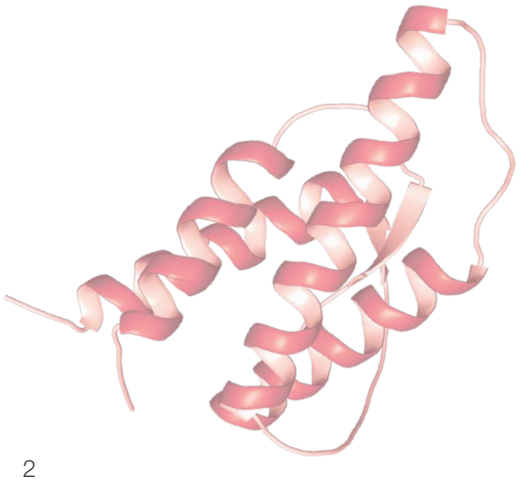
ProcartaPlex multiplex immunoassays are bead-based assays for protein detection using Luminex xMAP technology. ProcartaPlex assays are based on the principles of a sandwich ELISA, using two highly specific antibodies binding to different epitopes of one protein. See page 6 for more details.

QuantiGene Plex assays for RNA quantitation

The QuantiGene Plex assay also utilizes Luminex xMAP technology and is a high-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. See page 10 for more details.

Comparison of different multiplex assay platforms

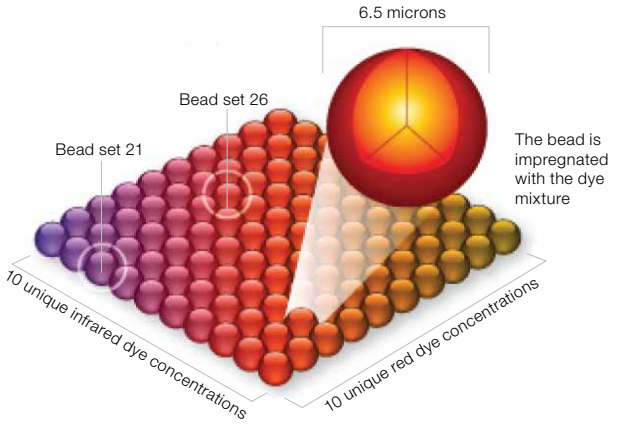
Assays	Analyte measured	Plex level	Targets available	Customization available
ProcartaPlex multiplex immunoassays	Protein	80	>600	Yes
QuantiGene Plex assays	RNA	80	>22,500	Yes



Luminex xMAP technology

How Luminex xMAP technology works

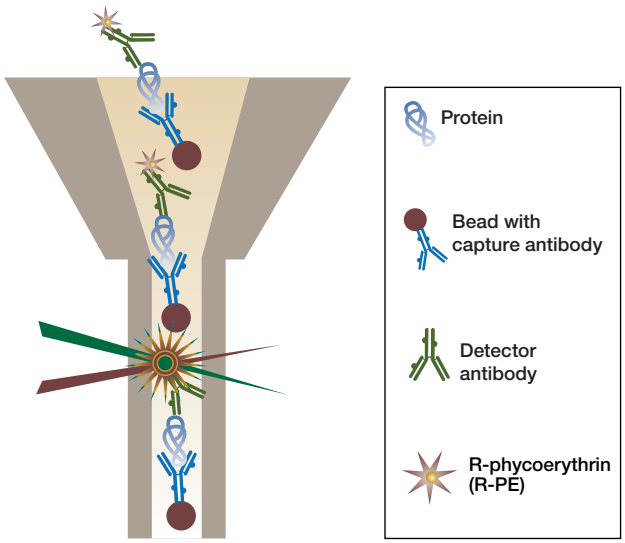
Sets of magnetic microspheres or beads are color-coded with specific ratios of red and infrared fluorophores. Each bead is coated with a reagent specific to a particular biomarker, allowing the capture and detection of several specific biomarkers from a single sample. Lasers in the compact analyzer excite 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.



How Luminex assays work

ProcartaPlex assays utilize Luminex xMAP magnetic bead technology, which uses Luminex® MagPlex® superparamagnetic 6.5-micron microsphere beads with a magnetic core. The beads are internally dyed with precise proportions of red and infrared fluorophores resulting in different spectrally unique microspheres, which are identified by the Luminex xMAP instruments. The conjugation of a specific monoclonal antibody to a distinct bead allows for analysis of multiple analytes in a single well. In the Luminex xMAP instrument, one laser (red) classifies the bead type to determine the analyte that is being detected while a second laser (green) determines the magnitude of the bound analyte (PE-derived signal). **See page 6 for more details**.

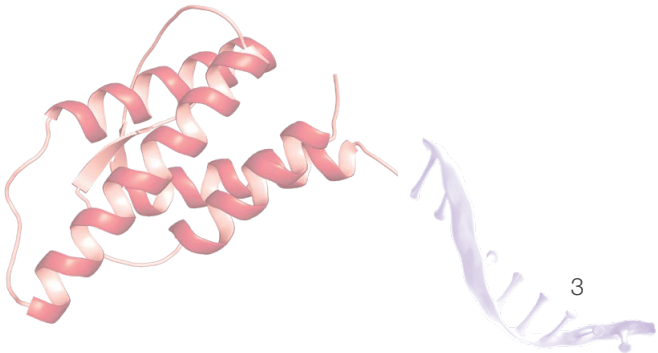
QuantiGene Plex assays also use Luminex xMAP magnetic beads, but instead of using antibodies, branched DNA (bDNA) technology is applied. This enables signal amplification for direct measurement of RNA transcripts. **See page 10 for more details**.



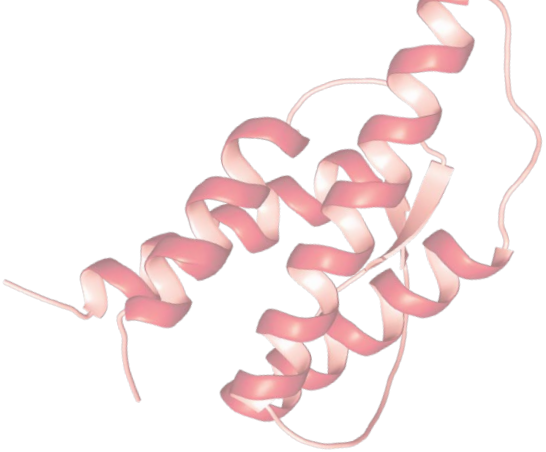
"The Luminex multiplexing platform is my go-to system as it provides rapid results that are both accurate and affordable, allowing for disease characterization, pathway detection, and biomarker discovery."

—Douglas D. Fraser, MD, PhD, FRCPC

Learn more at **thermofisher.com/luminex**



Luminex instrumentation



The Luminex family of instruments includes options for various multiplexing capabilities, throughputs, and read times. All instruments are capable of reading commercially available multiplex assays for protein and nucleic acid applications, which allow for multiplexing up to 80 biomarkers simultaneously. That's why Luminex instruments, paired with ProcartaPlex and QuantiGene assays, create an all-in-one platform for studying both proteomics and genomics.

Compare the multiplexing systems shown below to determine which Invitrogen™ Luminex® system is right for your laboratory's needs.

	Luminex® xMAP® INTELLIFLEX™ DR-SE System	Luminex® xMAP® INTELLIFLEX™ System	Luminex® FLEXMAP 3D® Instrument System	Luminex 200™ Instrument System
Applications	Protein and nucleic acid analysis			
Cat. No.	APX2021	APX2020	APX1342	APX10031
Multiplex capacity	Up to 500 targets Up to 2 parameters per analyte	Up to 500 targets	Up to 500 targets	Up to 100 targets
Read time (96-well plate)	~20 minutes	~20 minutes	~20 minutes	~40 minutes
Read time (384-well plate)	~75 minutes	~75 minutes	~75 minutes	N/A
Dynamic range	≥5.5 logs (RP1) ≥4.5 logs (RP2)	≥5.5 logs (RP1)	≥4.5 logs (RP1)	≥3.5 logs (RP1)
Microtiter plate	96- and 384-well	96- and 384-well	96- and 384-well	96-well
Dimensions	58.4 cm (23 in.) W 61 cm (24 in.) D 76.2 cm (30 in.) H	58.4 cm (23 in.) W 61 cm (24 in.) D 76.2 cm (30 in.) H	58.4 cm (23 in.) W 63.5 cm (25.7 in.) D 45.7 cm (18 in.) H	64 cm (25.25 in.) W 60 cm (23.5 in.) D 32.5 cm (12.5 in.) H
Touchscreen	•	•	N/A	N/A
Automated startup	•	•	N/A	N/A
Reporter laser	532 nm (green) and 405 nm (violet)	532 nm (green)	532 nm (green)	532 nm (green)
Dual reporter readout	•	N/A	N/A	N/A
Barcode reader for calibration and verification reagents	•	•	N/A	N/A
Analysis software	Data analysis apps for ProcartaPlex and QuantiGene Plex assays on the Thermo Fisher™ Connect Platform		Luminex® xPONENT® software (pre-installed), compatible with ProcartaPlex and QuantiGene Plex analysis apps on the Thermo Fisher Connect Platform	

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

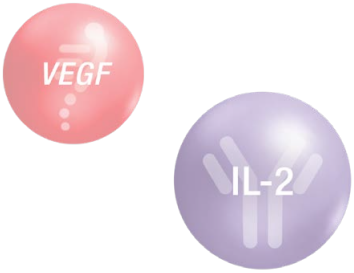
Learn more at **thermofisher.com/luminex**

Find out which instrument suits your needs and request a quote at

thermofisher.com/luminexinstruments

Combine workflows without compromising quality

Multiplex assays for gene and protein analysis



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 protein or RNA targets in single samples in either 96-well or 384-well format.

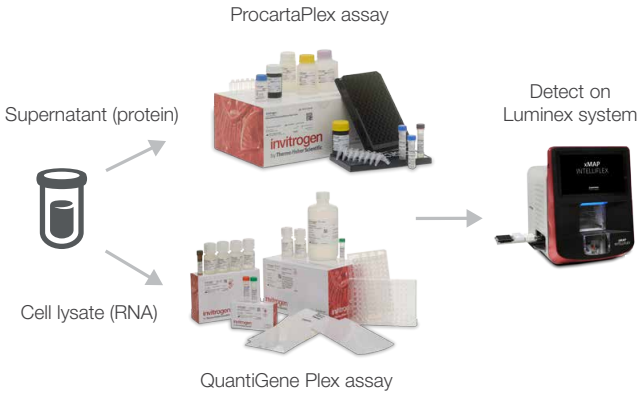


Figure 1. Up to 80 protein and 80 RNA targets can be measured in a single sample in 96-well or 384-well format.

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	80 proteins	80 RNA targets
Formats	96- and 384-well	
Sample types	Serum, plasma, cell culture supernatant (CCS), cerebral spinal fluid (CSF), cell lysates	RNA; cell and blood lysates; tissue and FFPE homogenates
Species	Human, mouse, rat, canine, porcine, non-human primate (NHP)	All
Compatible Luminex instruments	xMAP INTELLIFLEX Systems FLEXMAP 3D System Luminex 200 System MAGPIX System	
Sample volume	6.3–50 µL	20–80 µL
Customizable	Yes	Yes

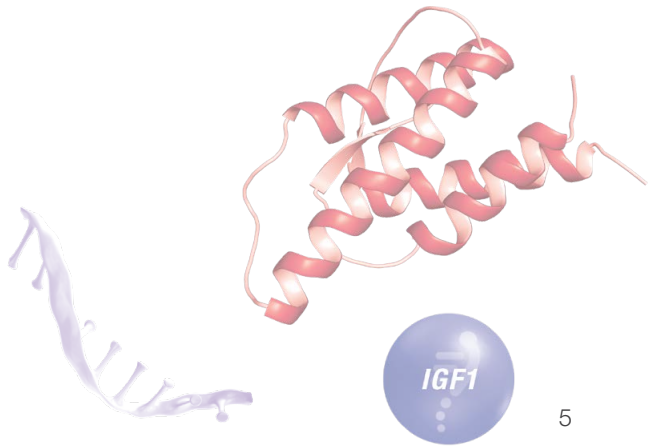
Methods

Multiplexing protein and gene level measurements on a single Luminex platform

White paper:

Multiplexing protein and gene level measurements on a single Luminex platform

To read the full publication, visit thermofisher.com/luminex



ProcartaPlex multiplex immunoassay

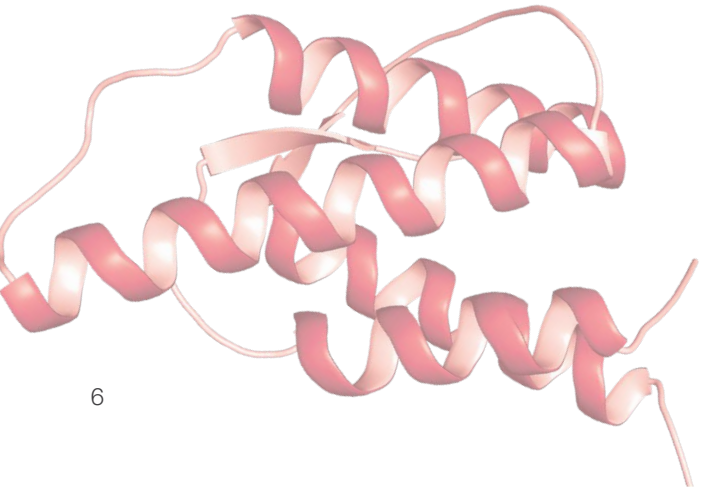
Profile more biomarkers with less sample

ProcartaPlex multiplex immunoassays use Luminex xMAP technology, which enables the simultaneous detection and quantitation of up to 80 protein targets in a single 6.3–50 µL sample—from plasma, serum, cell culture supernatants, cell lysates, 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 amount of time that it takes to perform a traditional sandwich ELISA (Figure 2).

ProcartaPlex multiplex immunoassays are available in preconfigured panels, or you can create your own unique panel through our flexible mix and match panel offering. Each panel undergoes stringent quality control and includes all necessary reagents to perform the assay.

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

Get information regarding characteristic assay details and individual analytes at **thermofisher.com/procartaplex**



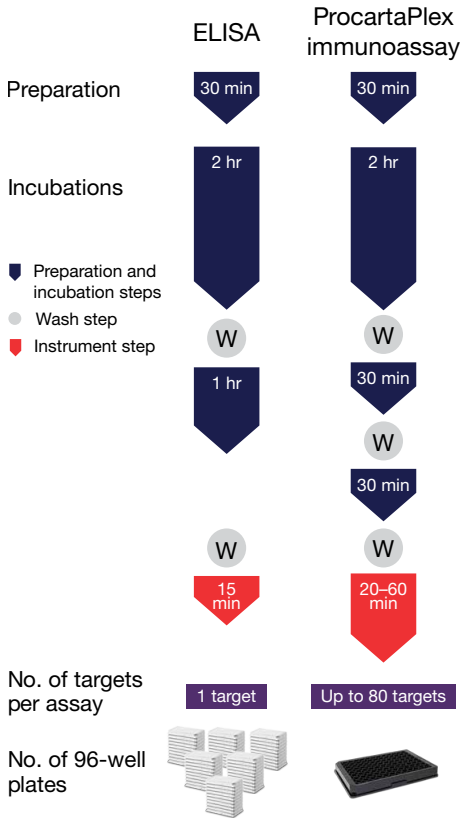


Figure 2. Workflow comparison of a conventional precoated ELISA to a ProcartaPlex multiplex immunoassay. A ProcartaPlex multiplex immunoassay can provide the same information as up to 80 individual ELISAs.

Assay specification comparison of a conventional ELISA and a ProcartaPlex multiplex immunoassay.

	ELISA	ProcartaPlex assay
Best for	Validation	Screening and verification
Time-to-result	4 hr	4.5 hr
Hands-on time	80 min	80 min
Wash steps	Yes, multiple	Yes, multiple
Multiplexing	No	Yes, up to 80 targets
Dual reporter	No	Yes
Sample volume	10–100 µL	6.3–50 µL
Analytical sensitivity	<10 pg/mL	<10 pg/mL
Dynamic range	<5–500 pg/mL	<5–20,000 pg/mL
Readout	HRP-TMB* (colorimetric)	R-PE* (RP1) PE (RP1)/Brilliant Violet* (RP2) (fluorescent)
Instrument	Microplate reader	Luminex instrument
Instrument read time	2 min	20–60 min

* HRP-TMB: horseradish peroxidase tetramethylbenzidine; R-PE: R-phycoerythrin; Brilliant Violet™ dye

Why choose ProcartaPlex multiplex immunoassays?

Flexibility
Get exactly the panel you want. More than 90% of ProcartaPlex multiplex assays can be combined with one another and allow for the creation of highly individualized and high-plex panels (≤ 80-plex).

Reproducible results
Each ProcartaPlex multiplex assay is manufactured in an ISO 9001–certified facility and undergoes rigorous QC testing to help ensure reproducibility and lot-to-lot consistency.

Scalability
Achieve reproducible performances regardless of plex size and correlate data from differently sized panels and simplex kits.

High specificity and sensitivity
All ProcartaPlex multiplex assays are extensively and individually tested for specificity and detection of native endogenous protein as well as for combinability, interference, and cross-reactivity.

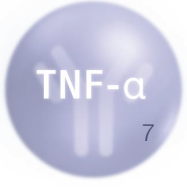
Save resources
ProcartaPlex multiplex assays require smaller sample input and less hands-on time than single-analyte assays, helping reduce time-to-results, cost, and labor.

Free analysis software
The ProcartaPlex™ Analyst App is free software available through the Thermo Fisher Connect Platform with 1 TB of free cloud storage.

ProcartaPlex multiplex assay options

Name	Description	Mixing required	Bead type	Base kit included
ProcartaPlex preconfigured panels	Predefined, biologically relevant, and disease-defined panels use magnetic beads for the quantitative multiplex analysis of up to 80 analytes in a single sample. Optimal performance and reproducibility is enabled through extensive testing for combinability, cross-reactivity, and interference.	No	Magnetic	Yes
ProcartaPlex mix and match panels	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
ProcartaPlex simplex kits	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 nontarget-specific reagents needed to perform the assay.	Yes	Magnetic	No
ProcartaPlex dual reporter assays	Preconfigured, ready-to-use, dual reporter panel that enables the measurement of two parameters of the same analyte on a single Luminex bead (e.g. phosphorylated and total form of signaling proteins).	No	Magnetic	Yes

Learn more at **thermofisher.com/procartaplex**



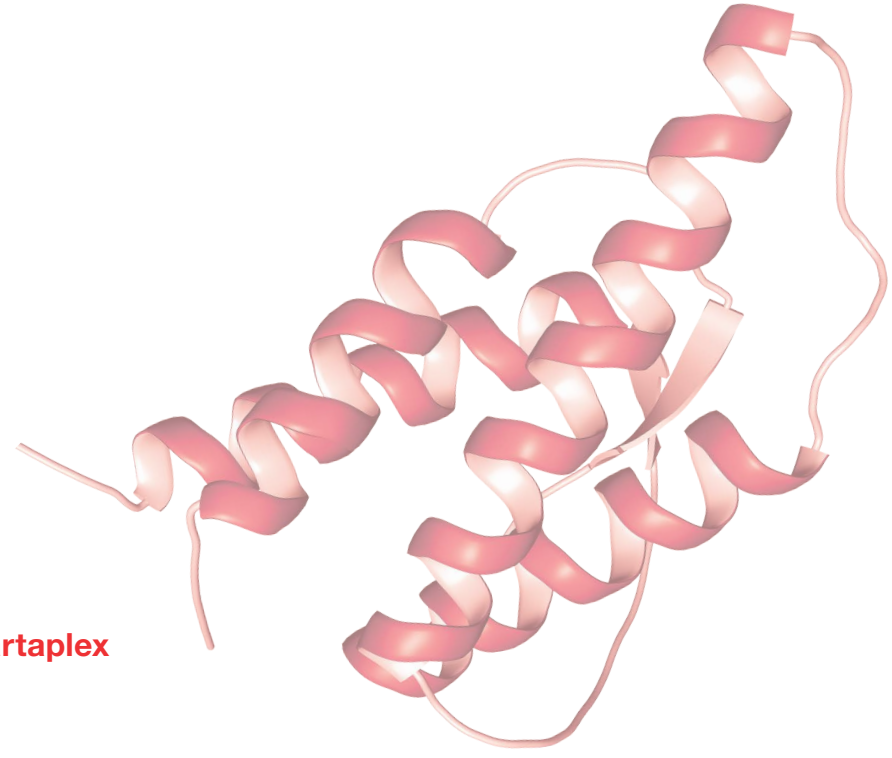
Product highlight: ProcartaPlex Human Immune Response Panel, 80plex

The Invitrogen™ ProcartaPlex™ Human Immune Response Panel, 80plex 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 high-plex panels (>50-plex) are excellent for initial biomarker screening. After, a more focused panel with key biomarkers can be used to refine your research. ProcartaPlex panels consistently deliver reproducible results regardless of plex size.

ProcartaPlex panel content

ProcartaPlex Human Immune Response Panel, 80plex							
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 alpha
bNGF	Eotaxin	GRO alpha	IL-18	IL-3	IP-10	MIG	TNF beta
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 alpha	TSLP
CCL25 (TECK)	Gal-3	IL-12p70	IL-21	IL-5	MCP-3	MIP-3 beta (CCL19)	TWEAK
CD30	G-CSF	IL-13	IL-22	IL-6	MCP-4 (CCL13)	MMP-1	VEGF-A



Learn more at **thermofisher.com/procartaplex**

Product highlight: ProcartaPlex signaling assays

Invitrogen™ ProcartaPlex™ signaling assays help unveil the complex interactions in studying cell signaling pathways by simultaneous measurement of up to eight signaling targets in a single well. These immunoassays are available in our ProcartaPlex assay format compatible with any Luminex xMAP instrument, and in our novel ProcartaPlex dual reporter assay format (Figure 3).

The innovative ProcartaPlex signaling dual reporter assays offer the unique capability to double your multiplex data by detecting two parameters of the same analyte leveraging the additional reporter channel of the Luminex xMAP INTELLIFLEX DR-SE System.

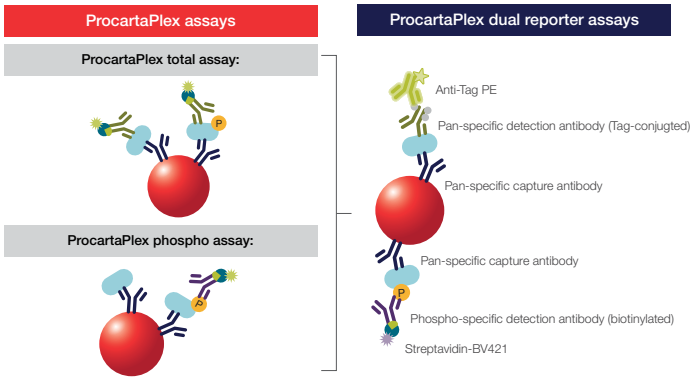
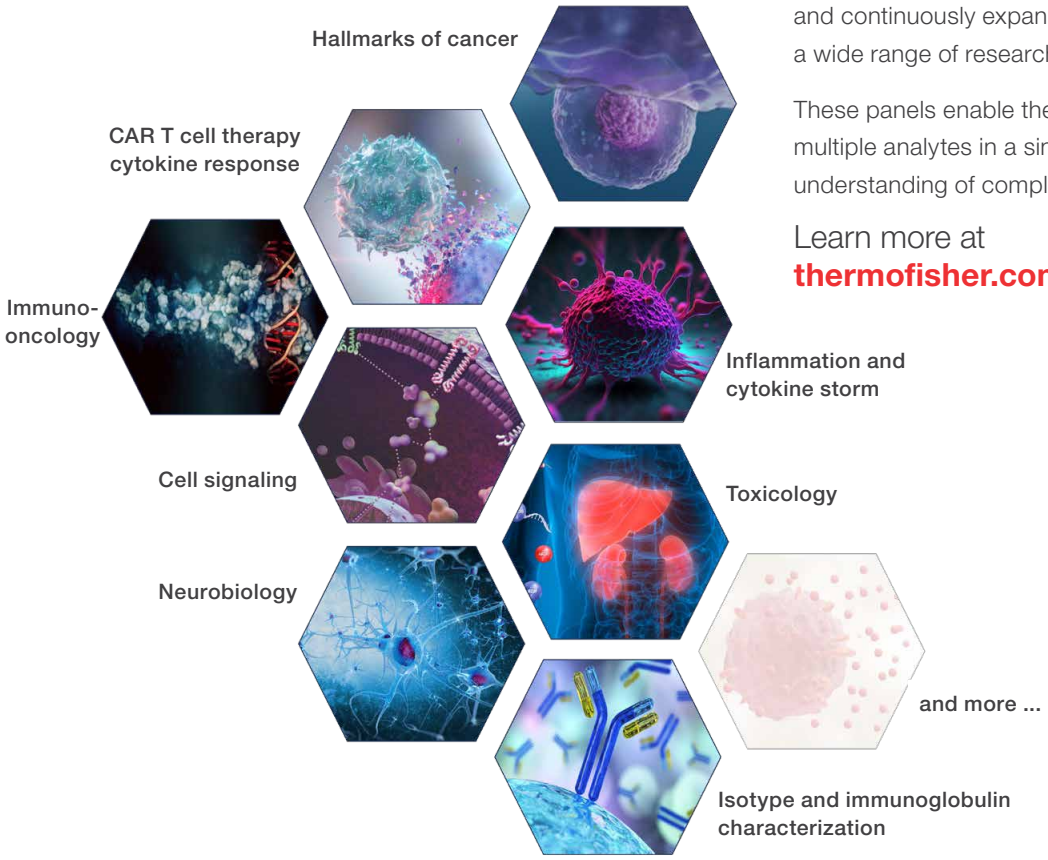


Figure 3. ProcartaPlex assay design. A ProcartaPlex assay enables measurement of either the phosphorylated or the total form of a signaling protein. The ProcartaPlex dual reporter assay allows measurement of both states simultaneously.

ProcartaPlex multiplex immunoassay supports a multitude of research areas



ProcartaPlex multiplex immunoassays offer an extensive and continuously expanding selection of panels for studying a wide range of research areas.

These panels enable the simultaneous detection of multiple analytes in a single sample and facilitate deeper understanding of complex biological systems.

Learn more at
thermofisher.com/immunoassayresearch

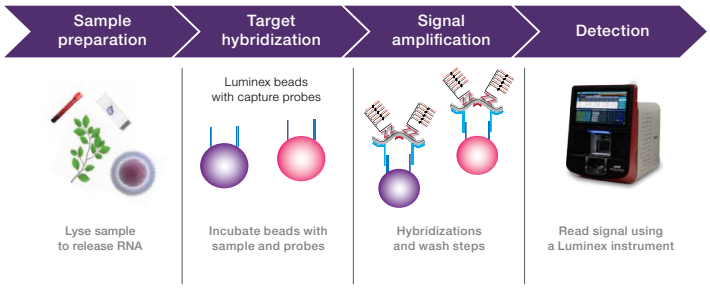
QuantiGene Plex assays

Accurate and precise RNA quantitation of up to 80 genes

QuantiGene Plex assays offer a powerful and efficient solution for gene expression analysis, providing accurate and reliable results in an easy four-step workflow.

QuantiGene Plex assays utilize Luminex xMAP technology, and combined with branched DNA technology, they enable:

- **True multiplexing**—measure up to 80 genes in a single well and save on precious sample and time
- **Flexibility**—select from over 22,500 verified genes to create customized panels or choose from our preconfigured offering
- **No need for RNA purification**—simply lyse and go
- **Compatibility with difficult samples types**—degraded and crosslinked RNA in formalin-fixed, paraffin-embedded (FFPE) tissues, and directly from blood samples



Multi-omics approach

Combine QuantiGene Plex assays with ProcartaPlex assays to get a more holistic picture of your sample by combining workflows on the same Luminex platform.

See details on page 5

QuantiGene Plex assay applications

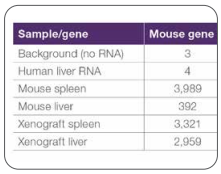
QuantiGene Plex assays for RNA profiling are exceptional for supporting drug discovery and development efforts, as well as translational and clinical research. The examples below highlight the capabilities and benefits of this powerful research tool.

Target discovery
Target identification and verification of cell-based microarrays

Lead optimization
High-throughput screening and secondary screening

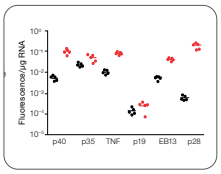
Preclinical studies
ADME and toxicology

Clinical research studies
Biomarker verification and clinical trials (FFPE tissues)

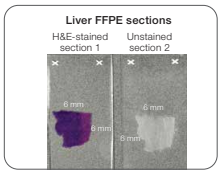


Sample/gene	Mouse gene
Background (no RNA)	3
Human liver RNA	4
Mouse spleen	3,989
Mouse liver	392
Xenograft spleen	3,321
Xenograft liver	2,959

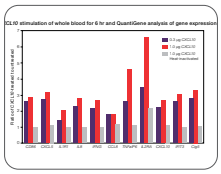
Patient-derived xenograft (PDX) models



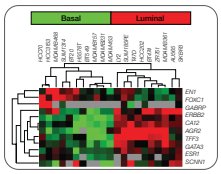
Verification of genes involved with inflammation



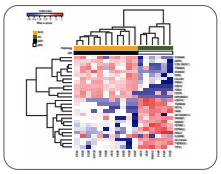
Verification of genes in FFPE samples



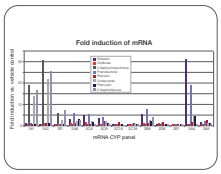
Expression analysis from whole human blood



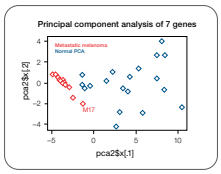
Tumor cell line characterization for drug compound screening



FFPE analysis of genes involved with cancer



CYP genes used with human hepatocytes



PCA analysis of genes involved with melanoma

Learn more at **thermofisher.com/quantigene**

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 the table decades of experience in automated incubation, laboratory robotics, and workflow scheduling. Our dedicated team of specialists provides automation support and expertise to help 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 are available 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.

Automation—not just for throughput

Automation can help 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**

ThermoFisher SCIENTIFIC

Custom services available

Assay development service

Custom assay development services are available for protein and gene targets that are not commercially available. You're leveraging years of assay development experience when you use our services. Let us help you save time so you can focus on what really matters—your research.

Benefits include:

- Dedicated project managers who work with you at every stage of your project
- Saving time and money, and avoiding the hassle of optimization and troubleshooting
- Confidence that you're getting an excellent assay

Sample testing service

Don't have a Luminex xMAP instrument? Not sure if it is the right platform for your investment? Test the technology and see for yourself whether the data meet your expectations. Our service team of highly skilled technicians ensures delivery of high-quality data and results.

Benefits include:

- Confidence that your precious samples will be run by experts
- High-quality reports for your assay data
- Minimal investment needed to decide whether to invest in a full Luminex platform

Learn more about our custom services at **thermofisher.com/immunoassayrequests**

Ordering information

	Description	Cat. No.
	**Luminex xMAP INTELLIFLEX instruments and accessories**	
	Luminex xMAP INTELLIFLEX DR-SE System	APX2021
	Luminex xMAP INTELLIFLEX System	APX2020
	INTELLIFLEX Calibration Kit	IFXCALK20
	INTELLIFLEX Performance Verification Kit	IFXPVERK20
	**Luminex FLEXMAP 3D instrument and accessories**	
	Luminex FLEXMAP 3D Instrument System	APX1342
	Luminex FLEXMAP 3D Calibration Kit	F3DCALK25
	Luminex FLEXMAP 3D Performance Verification Kit	F3DPVERK25
	**Luminex 200 instrument and accessories**	
	Luminex 200 Instrument System	APX10031
	Luminex 200 Calibration Kit	LX2RCALK25
	Luminex 200 Performance Verification Kit	LX2RPVERK25
	**Sheath fluid**	
	xMAP Sheath Fluid Plus	4050021
	xMAP Sheath Concentrate PLUS, RUO	4050023
	Assays for a Luminex system	
	ProcartaPlex multiplex immunoassay	thermofisher.com/procartaplex
	QuantiGene Plex gene expression assay	thermofisher.com/quantigene

Learn more at **thermofisher.com/luminex**

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