Panomics

Luminex® Assays

High-throughput Multiplex Bead Based Assays

Luminex assays are based on xMAP® technology (multi-analyte profiling beads) enabling the detection and quantitation of multiple RNA or protein targets simultaneously. The xMAP system combines a flow cytometer, fluorescent-dyed microspheres (beads), lasers and digital signal processing to efficiently allow multiplexing of up to 100 unique assays within a single sample.

Panomics has an aggressive program for the development of a broad range of assays for the Luminex platform and similar instruments based on the xMAP technology. Currently we have hundreds of RNA targets available in 3–30 plex assays using our using our QuantiGene Plex Reagent Systems, with new targets being added weekly. Our Procarta® Human, Mouse and Rat Assays can quantitatively measure cytokines and chemokines from a variety of sample sources, including serum and plasma. We've recently expanded our family of assays designed to measure protein expression and monitor protein modifications/activation states in diverse matrices.

We are also proud to be one of the few Luminex Certified Developers. Luminex have recognized us as having both unique and extensive assay development capabilities. We are happy to discuss your specific needs and develop and validate your assay to the same exacting standards as our commercially available assays.

Luminex Assays from Panomics

Gene Expression—quantitatively measure up to 30 different RNA transcripts

Transcription Factor—Profile up to 40 different active TFs from a single sample

Cytokine/Chemokines—quantitatively measure up to 33 different secreted proteins in serum, plasma or cell culture supernatant in human and mouse samples

SH2 Domains—Profile phosphotyrosine interactions with 30 SH2 protein binding domains

Custom Built Assays—If you can't find what you're looking for, we can custom design and build your assay. As one of the few Luminex Certified Developers, you can be assured of quality and a speedy response.

QuantiGene Plex 2.0—Taking Multiplexed Gene Expression to the Next Level

QuantiGene Plex 2.0 and Luminex

QuantiGene® Plex offers high reproducibility and ease-of-use that make it the perfect assay to bridge the technology gap when studying many genes in a limited number of samples and studying a few genes in a large number of samples. With QuantiGene Plex, researchers can easily perform multiplexed analyses from rare or volume-limited samples and can compare results across different samples, experiments and laboratories. Profiling many genes simultaneously in a single reaction directly from cultured cell or whole blood lysates, or fresh, frozen or FFPE tissue homogenates, can be accomplished without the need for RNA purification, reverse transcription, or amplification.

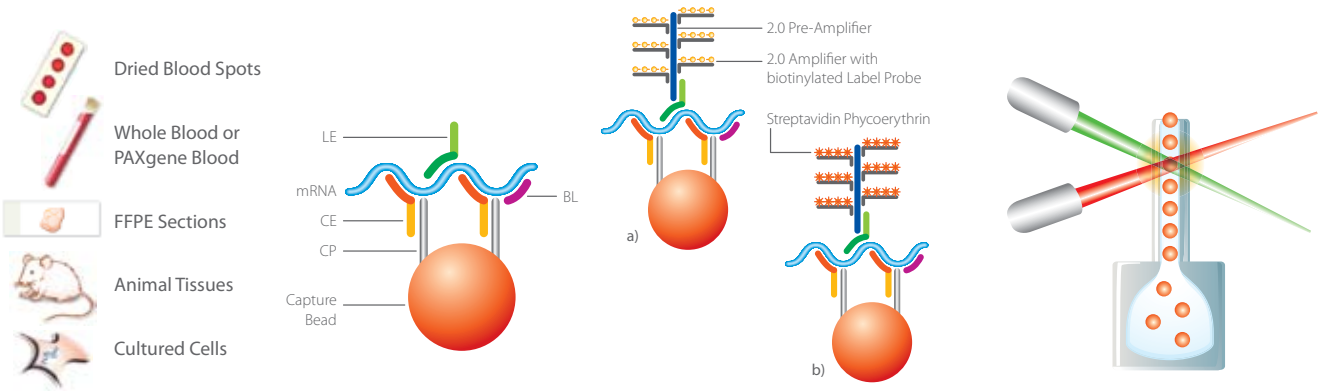
QuantiGene Plex 2.0 assays combine branched DNA (bDNA) signal amplification technology and xMAP (multi-analyte profiling) beads to enable simultaneous quantification of multiple RNA targets directly from cultured cell or whole blood lysates; fresh, frozen or formalin-fixed, paraffin-embedded (FFPE) tissue homogenates; or purified RNA preparations. Clinically proven Branched DNA technology is a sandwich nucleic acid hybridization assay that provides a unique approach for RNA detection and quantification by amplifying the reporter signal rather than the target sequence. By measuring the RNA at the sample source, the assay avoids variations or errors inherent to extraction and amplification of target sequences.

Assay Specifications

Limit of Detection	≤ 2,000 transcripts/assay well
Limit of Quantitation	≤ 5,000 transcripts/assay well
Linear Dynamic Range	≥ 3 logs
Assay CV	≤ 15% intra-assay; ≤ 20% inter-assay
Compatible Sample Types	Cultured cells, whole blood, PAXgene blood or dried blood spots, fresh/frozen tissues, FFPE samples, purified RNA
Assay Format	96-well plate
Targets/well	3–30

QuantiGene Plex Applications

- **Prospective/retrospective studies using whole blood or FFPE samples**
- **Biomarker validation**
- **Predictive toxicology**
- **Microarray validation**
- **Secondary screening**
- **RNAi knockdowns and monitoring of "off-target" effects**



Step 1: Release Target RNA

Cells are lysed to release RNA.

Step 2: Target RNA Capture

Specific mRNA transcripts are captured to their respective beads through a Capture Extender (CE) Capture Probe (CP) interaction during an overnight hybridization at 54°C.

Step 3: Signal Amplification

a) Sequential hybridization of the 2.0 Pre-Amplifier, 2.0 Amplifier and biotinylated Label Probe, respectively, for an hour at 50°C.

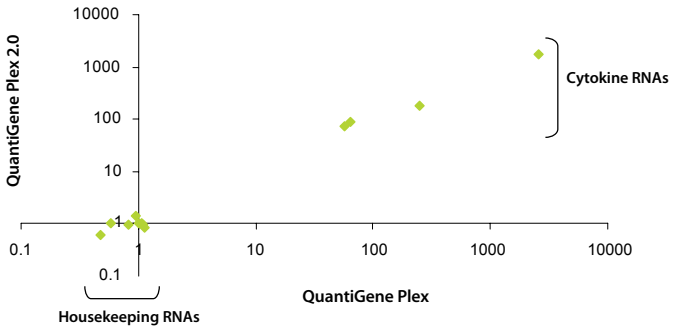
b) Binding with Streptavidin-conjugated Phycoerythrin (SAPE) at room temperature for 30 minutes.

Step 4: Detection

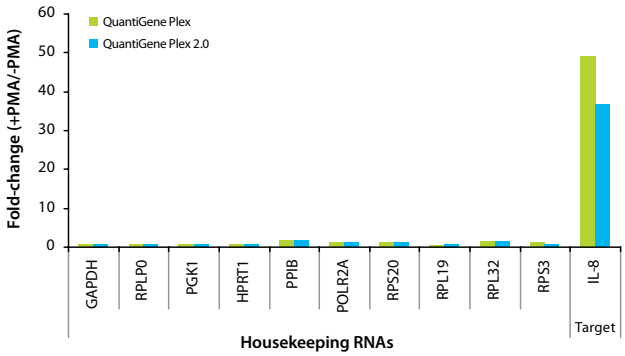
The sample is analyzed on a Luminex* instrument. The level of SAPE fluorescence is proportional to the amount of mRNA transcripts captured by the bead.

** Bio-Plex suspension array system or other Luminex-based array systems.*

Demonstrated Performance with Clinically Relevant Sample Types



Values in the graph demonstrate the expected induction of 4 cytokine RNAs in 10 µL of whole blood samples treated with LPS. No significant changes were detected in the expression of 8 housekeeping RNAs. Data from QuantiGene Plex 1.0 and QuantiGene Plex 2.0 assays had a correlation coefficient of 0.9995.



Values in the graph demonstrate the expected induction of IL-8 in FFPE preparations of HeLa cell pellets treated with PMA. No significant changes were detected in the expression of 10 housekeeping RNAs. Data from the QuantiGene Plex 1.0 and QuantiGene Plex 2.0 assays had a correlation coefficient of 0.9999.

Using QuantiGene Plex 2.0 in High Throughput Applications

Many potential drugs that specifically target a particular protein considered to underlie a given disease have been found to be less effective than hoped, or to cause significant side effects. The intrinsic robustness of living systems against various perturbations is a key factor that prevents such compounds from being successful.

By including screening measurements in a more integrated manner using chemical genomic approaches, i.e. associated pathway elements, dose response, "off target" targets, the likelihood of identifying more robust compounds (SME's) or biologicals that have a higher chance of ultimate success will increase significantly. At the same time promising candidates that would have ultimately failed at a later stage in the development process will be identified during screening, enabling higher attrition rates supporting the ultimate goal of "failing faster".

QuantiGene Plex 2.0 is ideally suited to deliver cost effective multiplex data for these new high value contextually relevant assays. Benefits to consider:

- **Target additional pathway elements not just primary genes of interest**
- **Get earlier indication of toxicology profiles and stress indicators**
- **Develop dose response profiles**
- **384 well or 96 well plate formats are supported**
- **Assay can be readily automated**
- **Our patent pending plex/plex methodology reduces cost and labor considerably**
- **Compatible with the Luminex HTS system**

Assay Highlights

Quantitatively measure multiple RNA targets simultaneously with unparalleled accuracy and precision

RNA quantitation directly from cultured cells, whole blood, or fresh, frozen or formalin-fixed, paraffin-embedded (FFPE) tissue

– No RNA purification
– No reverse transcription
– No target amplification

Simple Assay Workflow

Widely used in biomarker validation, microarray validation, predictive toxicology and secondary screening

QuantiGene Plex Publications

1. Gupta, A., et al., Role of protein C in renal dysfunction after polymicrobial sepsis. J Am Soc Nephrol, 2007. 18(3): p. 860-7.
2. Flagella, M., et al., A multiplex branched DNA assay for parallel quantitative gene expression profiling. Anal Biochem, 2006. 352(1): p. 50-60.
3. Zheng, Z., Y. Luo, and G.K. McMaster, Sensitive and quantitative measurement of gene expression directly from a small amount of whole blood. Clin Chem, 2006. 52(7): p. 1294-302.
4. Zhang, A., et al., Small interfering RNA and gene expression analysis using a multiplex branched DNA assay without RNA purification. J Biomol Screen, 2005. 10(6): p. 549-56.

Procarta Transcription Factor Plex—Luminex Assays for Transcription Factor Profiling

About Transcription Factors

Transcription Factors (TFs) are highly conserved proteins that bind to DNA and initiate transcription of a given gene. A single extracellular stimulus can trigger multiple signaling pathways, and these in turn can activate multiple TFs to mediate the inducible expression of target genes.

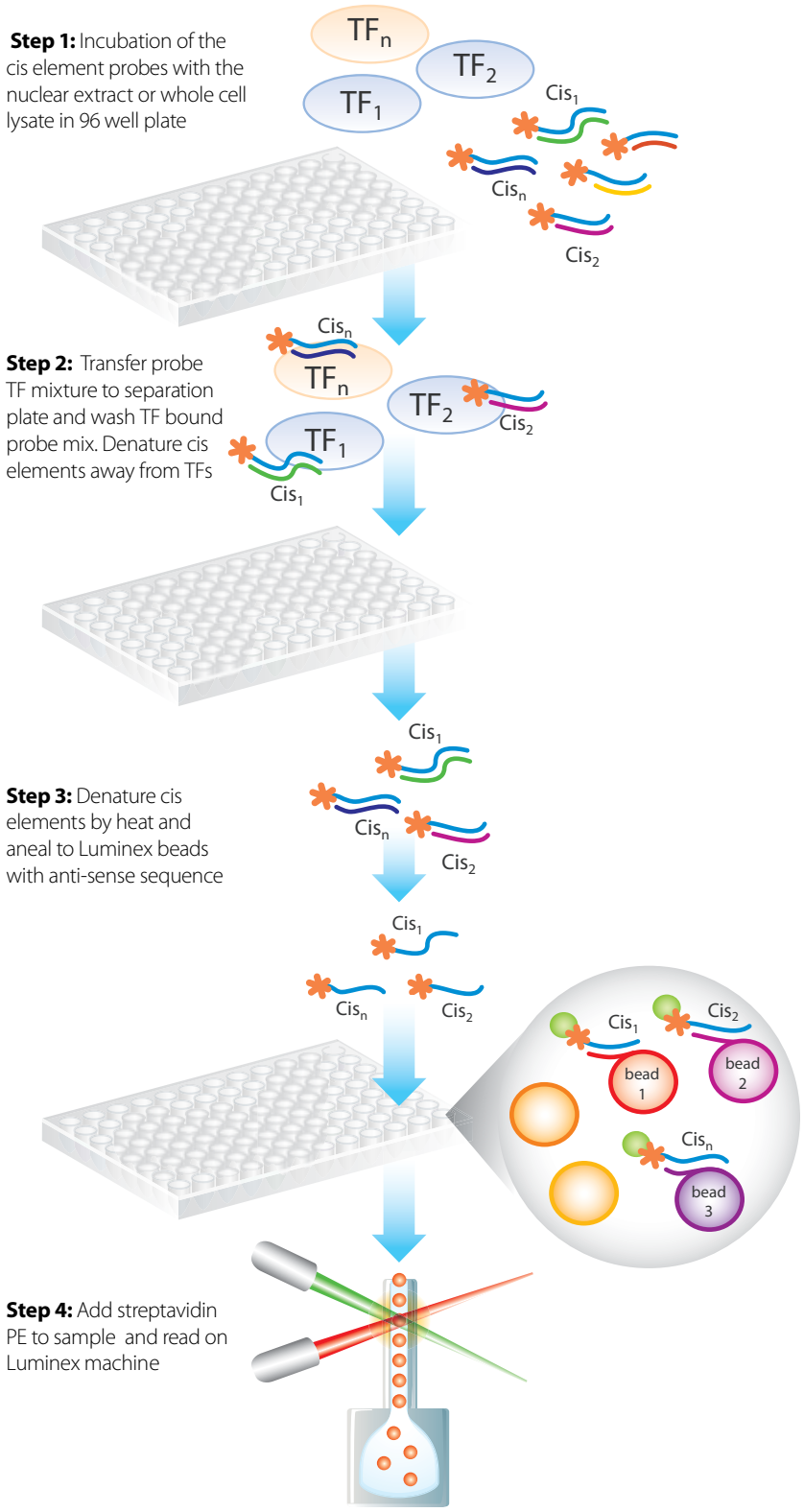
The Procarta TF Plex assay is a profiling assay for monitoring the activation of TFs. We have developed two panels (40-plex and 43-plex) which are Luminex based to profile and measure activated TFs. The 96-well plate format enables high-throughput profiling of the DNA binding activity of TFs in multiple samples with high sensitivity.

Key Applications

- **Profile the activities of multiple TFs upon a given drug stimulus**
- **Monitor off-target effects upon a given drug treatment**
- **Confirm cell signaling pathways using the TF Plex Assay**

How It Works

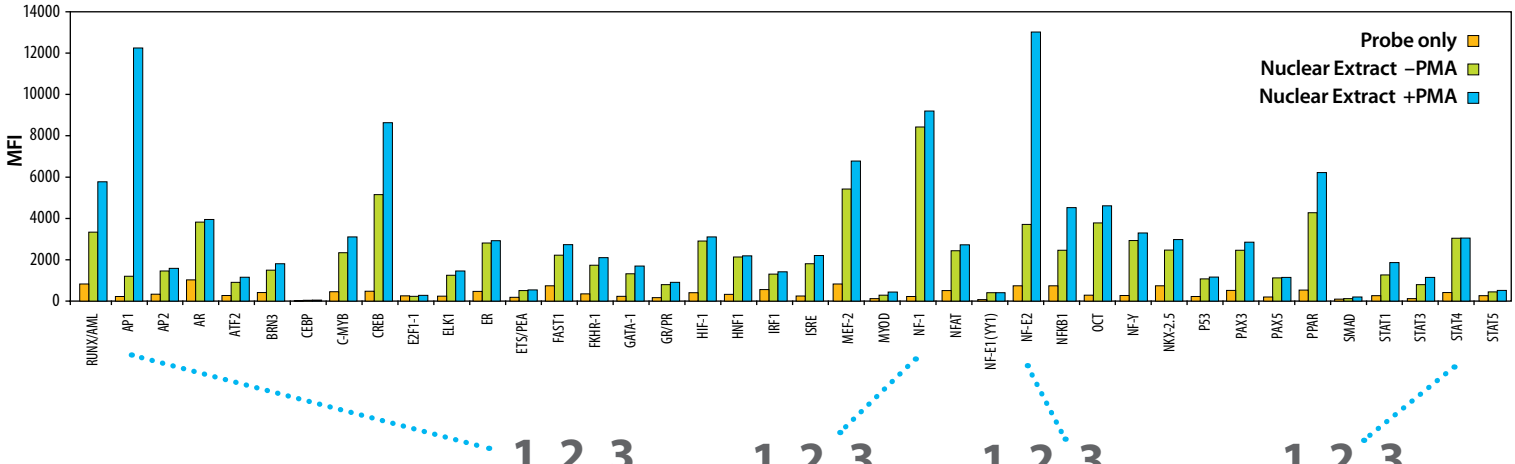
Our novel, Procarta TF assay allows for the profiling of multiple TFs from a variety of sample types including cell lysates and nuclear extracts. Up to 40 TFs can be analyzed in one well.



Create your own TF Plex Panel

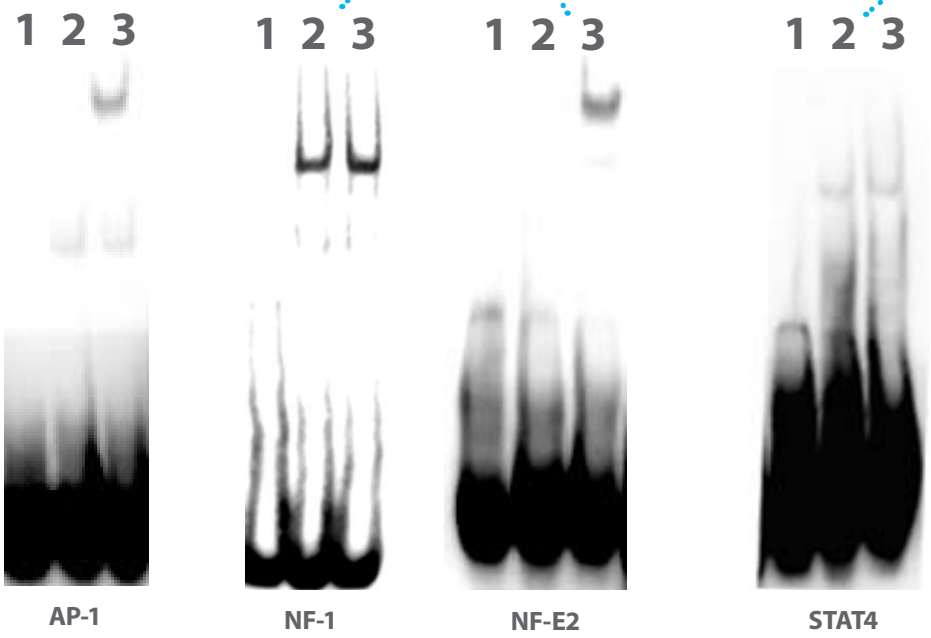
You have the option to either order the full 40 plex or choose TFs from either panel to create your own unique 3-39 plex.

TF 40-plex assay: Nuclear Extract from HeLa +/- PMA



Procarta TF Plex Assay Confirmed by EMSA

The activity of 40 different TFs were profiled using the Procarta Transcription Factor Plex Assay. Nuclear extracts were prepared from serum starved HeLa cells subsequently stimulated with PMA or a vehicle control for 4 hours. Extracts were used on the Procarta TF Plex assay and the EMSA Gel Shift assays.



Procarta TF Panel 1 (40 TFs)

RUNX/AML	ELK-1	ISRE	OCT
AP-1	ER	MEF-2	p53
AP-2	ETS/PEA	MYOD	PAX-3
AR	FAST-1	NF-1	PAX-5
ATF-2	FKHR-1	NFAT	PPAR
BRN-3	GATA-1	NF-E1/YY1	SMAD
CEBP	GR/PR	NF-E2	STAT-1
C-MYB	HIF-1	NFkB	STAT-3
CREB	HNF1	NKX-2.5	STAT-4
E2F-1	IRF-1	NF-Y	STAT-5

Visit our website to see the most current list.

Procarta TF Panel 2 (43 TFs)

ALF-1/TAL-1	ELF-1	KPF-1	PUR-1
ANTIOXIDENT RE	EVI-1	LF-A1	RB
AP-1	GAG	LVF	SIE
AP-4	GFI-1	MRE	SRE
CCAAT	H4TF	MTF	SRY
CDP	HAS+HBS	NEUROD1	TFE-3
CEF-1	HBS/XBP	NFkB	TR
C-MYC	HINF	NPAS2	TR(DR-4)
COUP-TF	HSF	PDX-1	TREF-1/2
E47	IKAROS	PIT-1	USF-1
EGR	XBP-1	XRE	

Create your own TF Plex Panel

You have the option to either order the full plex sets from Panel 1 or Panel 2 or choose TFs from within either panel to create your own plex set.

Procarta Quantitative, Multiplexed Cytokine/Chemokine Assays

Overview

Procarta Cytokine/Chemokine assays use the xMAP technology (mutli-analyte profiling beads) to enable the detection and quantitation of multiple protein targets simultaneously. The Procarta assay kits are compatible with all Luminex and Luminex-based instruments currently available.

Measure Protein and Gene Expression from the Same Sample Well

Panomics' Procarta Cytokine Assay Kits enable the profiling of up to 33 (check website for an updated list) different cytokines per reaction. When coupled with Panomics' QuantiGene Plex assays, both gene and protein expression can be quantitated from the same sample well (supernatant for protein quantitation and cell lysate for mRNA quantitation) enabling the parallel study of gene expression at the RNA and protein level.

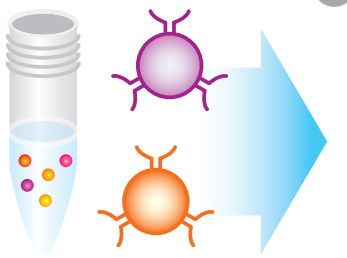
Procarta Cytokine Assay Kits

Procarta Cytokine assays simultaneously quantitate cytokines from diverse matrices in 3 hours with a sensitivity of 1 pg/mL/cytokine. Human, Mouse and Rat Cytokine/Chemokine Assay Kits are available in 1- and 10-plate sizes for fixed, off-the-shelf formats, or By Request, customer defined mix-to-order formats. By Request orders are processed and delivered in a pre-mixed ready to use format in ~1 week. Procarta Cytokine Assay Kits contain all the components required to process cell culture supernatant samples and include: pre-mixed, ready to use, antibody-conjugated beads; 96-well filter plate and holder; assay and wash buffers; sample buffer; pre-mixed, ready to use detection antibodies; Streptavidin-PE (SAPE) fluorescence detection reagent; and premixed antigen standards.

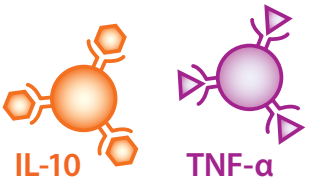
Procarta Standard Diluent Kits

Procarta Cytokine Standard Diluent Kits for plasma and serum, sold separately, contain a single component, species/matrix-specific Standard Buffer, for preparation of antigen standards and dilution of experimental samples (if required). Panomics' Procarta Cytokine Standard Diluent Kits are designed for use with Procarta Cytokine Assay Kits. Using the appropriate Standard Buffer ensures optimal recovery and sensitivity of the cytokines being analyzed in a serum or plasma matrix.

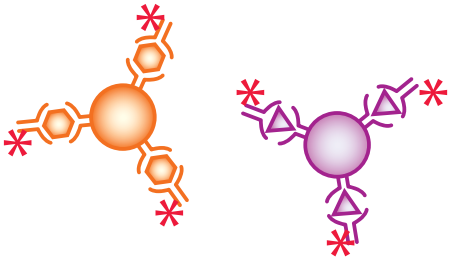
How It Works



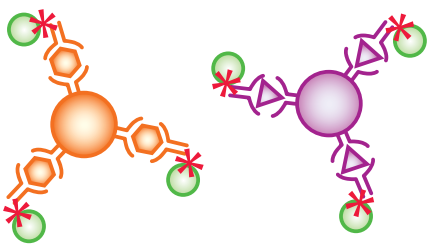
1 Incubate your sample with the antibody-conjugated beads for 30 minutes



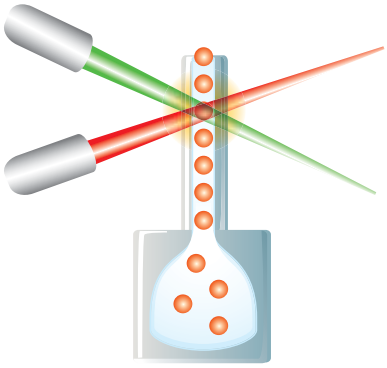
2 Add detection antibody and incubate for 30 minutes

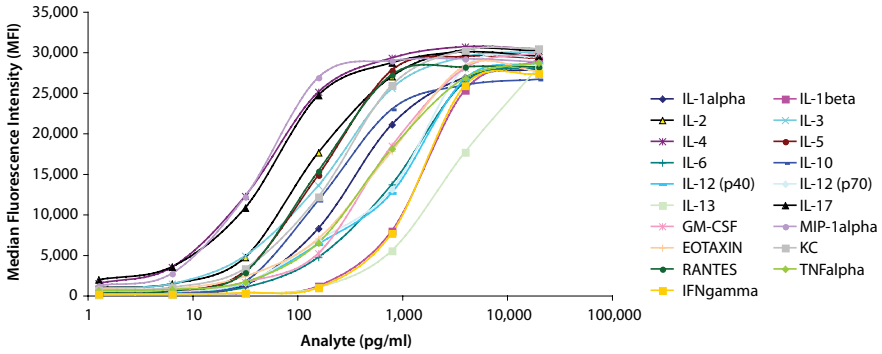


3 Add SAPE and incubate for 30 minutes



4 Detect interactions on a Luminex instrument





Standard Curves were generated in cell culture media using Panomics' 19-plex Procarta Mouse Cytokine Assay Kit. Each analyte has a sensitivity of 10 pg/mL or less and an assay range over 3 logs.

Assay Highlights

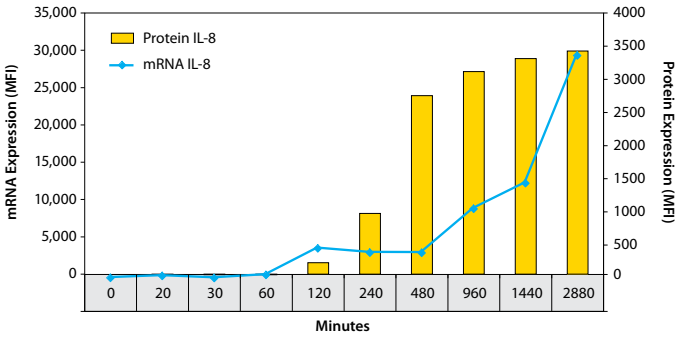
Mix and match to create your own plex set from Human, Mouse and Rat analytes from the list below

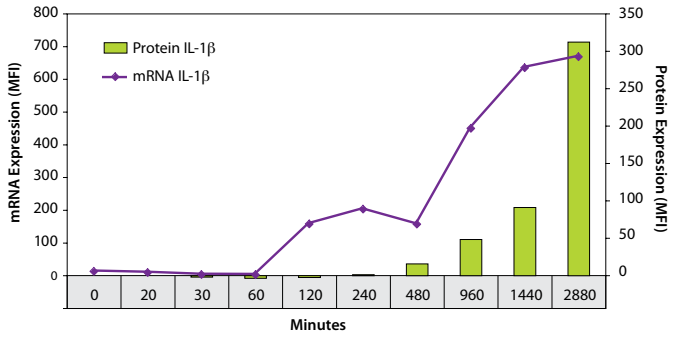
Complete assay in less than 3 hours

Reagents are supplied at 1X concentration and **ready to use**

Quantitative measurements from cell culture supernatants, serum or plasma samples

Minimal sample required, only 25 µL





Simultaneous analysis of protein and gene expression from the same sample. Human histocytic lymphoma cells, U-937, were treated with 1 µg/mL of LPS. At various timepoints, cells culture supernatants samples were collected and the corresponding cells were lysed. The supernatants were analyzed for 20 different cytokines using Procarta Human Cytokine Assay. The cell lysates were analyzed for 30 different cytokines using QuantiGene Plex Reagent System. The results of protein and gene analysis of two cytokines, IL-8 and IL-1β, are shown above.

Human 33	
L-1-alpha	GM-CSF
L-1-beta	GRO-alpha
IL-2	iIFN-gamma
IL-4	IP-10
IL-5	LEPTIN
IL-6	MCP-1
IL-7	MCP-3
IL-8	MIG
IL-10	MIP-1-alpha
IL-12(p70)	MIP-2-alpha
IL-12(p40)	NGF
IL-13	PDGF-BB
IL-17	RANTES
ENA-78	TGF-beta
EOTAXIN	TNF-alpha
FGF-basic	VEGF
G-CSF	

Mouse 23	
L-1-alpha	GM-CSF
IL-1-beta	IFN-gamma
IL-2	KC
IL-3	MCP-1
IL-4	MCP-3
IL-5	MIP-1-alpha
IL-6	RANTES
IL-10	TNF-alpha
IL-12(p40)	VEGF
IL-12(p70)	
IL-13	
IL-17	
IP-10	
EOTAXIN	

Rat 9
IL-1-alpha
IL-1-beta
IL-6
I-CAM
KC
MCP-1
MIP-1-alpha
TNF-alpha
VCAM

Specifications

Sensitivity	1 pg/mL/cytokine
Precision	Average Inter-assay CV <10% Average Intra-assay CV <10%
Spike Recovery (Accuracy)	80-120%
Cross Reactivity	Negligible
Matrices	Cell culture supernatants, serum, plasma

Phosphotyrosine Profiling Using SH2 Domains

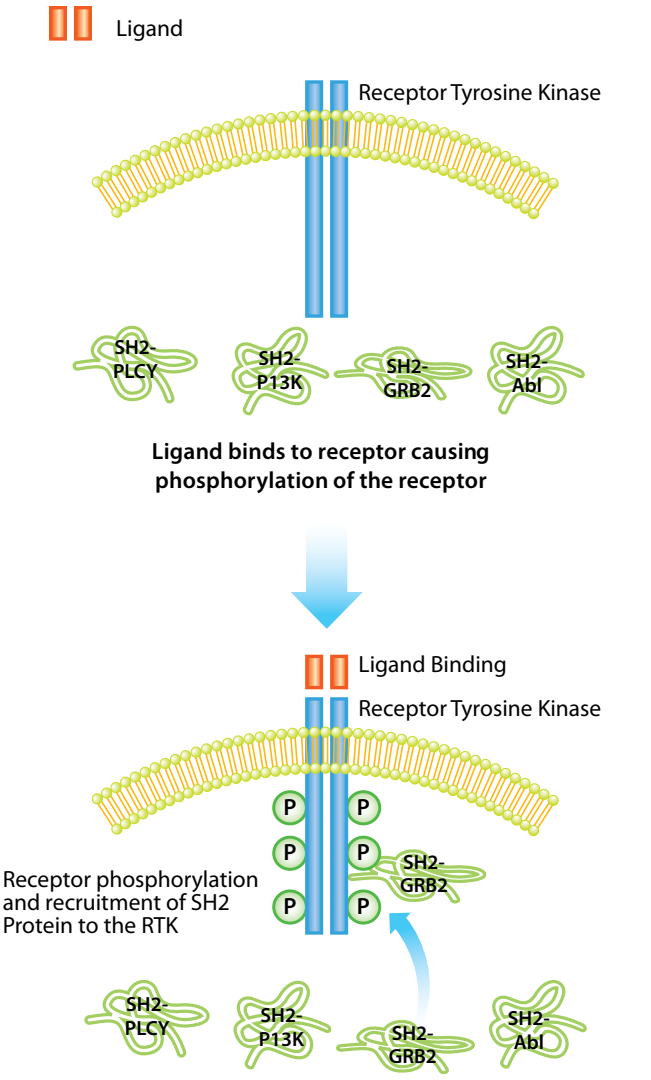
The SH2 Domain—a Key to Understanding Phosphotyrosine-Dependent Signal Transduction

SH2 domains are one of the many protein domain families that mediate protein-protein interactions in signal transduction. Like other domains, SH2 domains are defined by a conserved region of amino acid residues. The folding characteristics of this sequence of 100-amino acids allow these domains to specifically recognize and bind to phosphotyrosine-containing ligands.

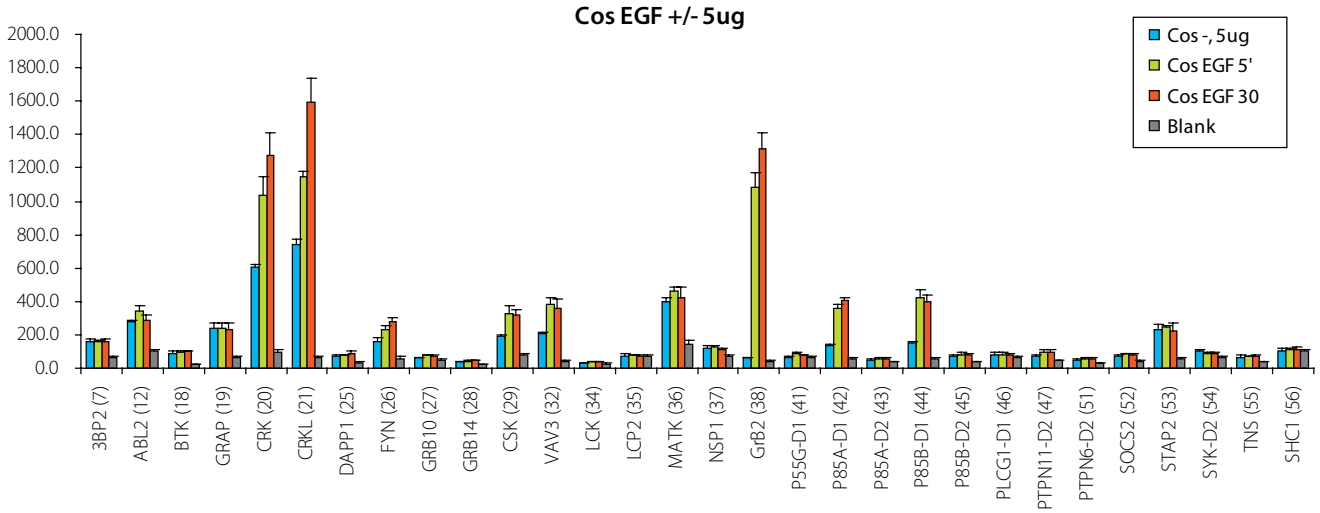
There are approximately 120 different SH2 domains that bind to 110 different proteins in the human genome. These protein-protein interactions involving phosphotyrosines, like those made possible by SH2 domains, are a primary means of recruiting signaling proteins, and thus play a major role in signal transduction.

SH2 domains can be found in enzymes, adaptor proteins, regulatory subunits of signaling proteins, scaffold proteins, transcription factors and oncogenic proteins. These proteins are integral to the signaling process because they act as adaptors between receptors and downstream signaling molecules, transmitting signals within cells and regulating the kinase activity of specific proteins.

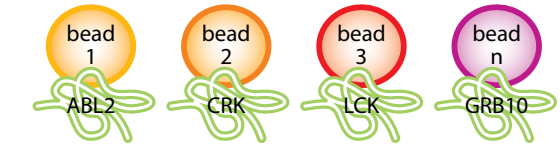
Protein phosphorylation is a major conduit of information for cellular responses, and defects in SH2 domain-dependent signaling are often directly or indirectly shown to be involved in human diseases.



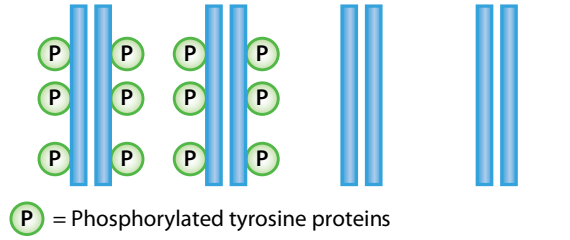
The Procarta SH2 Domain Plex assay is a 30 plex assay capable of profiling identifying differences of measuring SH2 proteins that have bound to phosphorylated tyrosine residues of proteins.



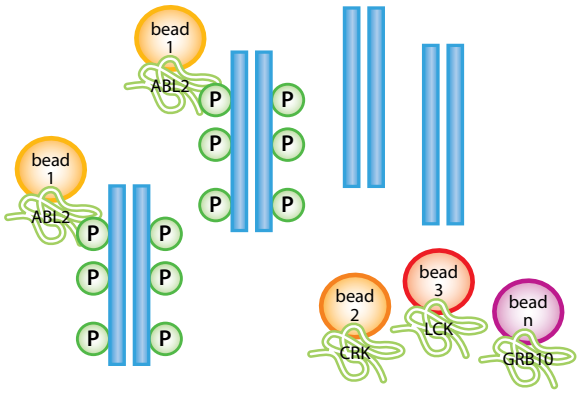
How It Works



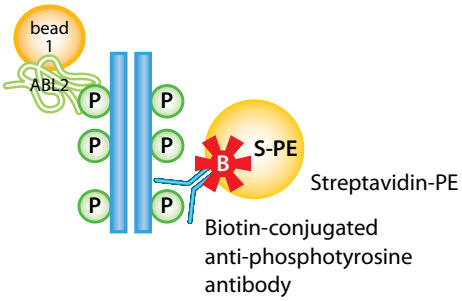
Panomics provides Luminex beads conjugated to SH2 proteins.



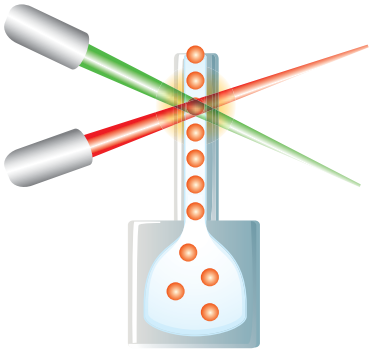
Treated and untreated cell lysates are prepared containing phosphorylated tyrosine kinases.



SH2 conjugated beads are added to the cell lysates and only the specific SH2 bead will bind to the phosphorylated receptor tyrosine kinases (RTKs).



Anti-phosphotyrosine antibody is added, followed by the addition of the Streptavidin PE. The complex is then analyzed on the Luminex System. The beads that do not have any bound RTKs will have little or no fluorescence.



Assay Highlights

Mix and match to create your own plex set

Complete assay in less than 4 hours

Reagents are supplied at 1X concentration and **ready to use**

Determine which SH2 domains bind phosphorylated proteins in a given pathway

Key Applications

SH2 Profiling

Peptide affinity screening

Drug binding screening

Phosphoprotein detection using specific antibody

Procarta SH2 Domain Plex

3BP2	CSK	P85B-D1
ABL2	VAV3	P85B-D2
BTK	LCK	PLCG1-D1
GRAP	LCP2	PTPN11-D2
CRK	MATK	PTPN6-D2
CRKL	NSP1	SOCS2
DAPP1	GRB2	STAP2
FYN	P55G-D1	SYK-D2
GRB10	P85A-D1	TNS
GRB14	P85A-D2	SHC1

Visit our website to see the most current list.

Create your own SH2 Plex Panel

You have the option to either order the full 30 plex or choose SH2s from the panel above to create a panel from 2-29 plex.

Luminex Technology Overview

Luminex's xMAP technology is built on proven, existing technology—flow cytometry, microspheres, lasers, digital signal processing and traditional chemistry—that have been combined in a unique way. Featuring a flexible, open-architecture design, xMAP technology can be configured to perform a wide variety of bioassays quickly, cost-effectively and accurately.

Luminex color-codes tiny beads, called microspheres, into 100 distinct sets. Each bead set can be coated with a reagent specific to a particular bioassay, allowing the capture and detection of specific analytes from a sample. Within the Luminex compact analyzer, lasers excite the internal dyes that identify each microsphere particle, and also any reporter dye captured during the assay. Many readings are made on each bead set, further validating the results. In this way, xMAP technology allows multiplexing of up to 100 unique assays within a single sample, both rapidly and precisely.

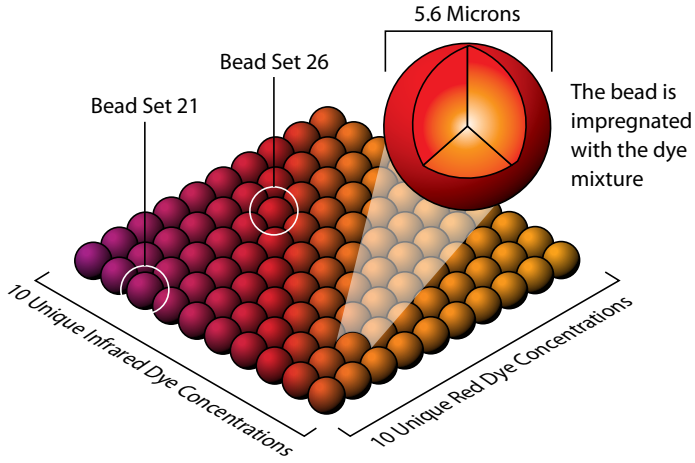
Here's How It Works

The Luminex System is a flexible analyzer based on the principles of flow cytometry that is designed to meet the needs of any size research laboratory. The system enables you to multiplex (simultaneously measure) up to 100 analytes in a single microplate well, using very small sample volumes. At Panomics though, we offer multiplexed solutions of up to 40 different analytes in a single well. The system delivers fast and cost-effective bioassay results on many assay formats that Panomics offers which include: gene expression, transcription factor profiling, cytokine profiling and SH2 Domain profiling.

The Luminex System is the combination of three core xMAP technologies. The first is xMAP microspheres, a family of 100 fluorescently dyed 5.6 micron-sized polystyrene microspheres that act as both the identifier and the solid surface to build the assay. The second is a flow cytometry-based instrument, the Luminex analyzer, which integrates key xMAP detection components such as lasers, optics, advanced fluidics and high-speed digital signal processors. The third component is the assays that are designed around the microspheres.

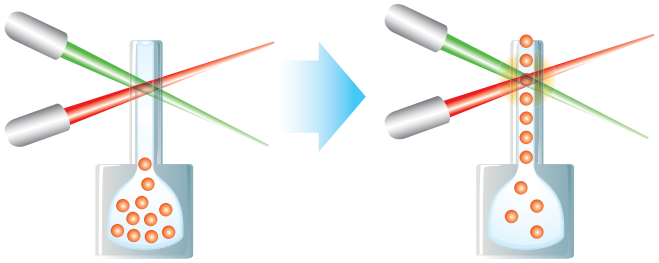
xMAP Technology

The xMAP technology uses 5.6 micron polystyrene microspheres which are internally dyed with red and infrared fluorophores. Using different amounts of the two dyes for different batches of microspheres, up to 100 different microsphere sets can be created. Each bead is unique with a spectral signature determined by it's red/infrared dye mixture. The bead is filled with a specific known ratio of the two dyes. As each microsphere carries a unique signature, the xMAP detection system can identify to which set it belongs. Therefore, multiplexing up to 100 tests in a single reaction volume is possible.

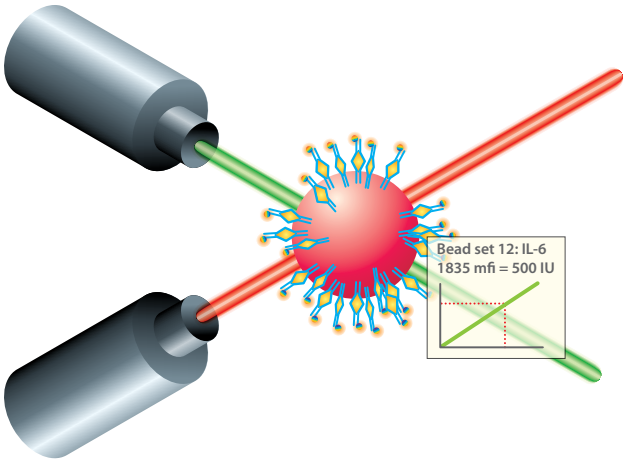


Luminex Reader Design

The Luminex reader combines two lasers, fluidics, and real-time digital signal processing to distinguish up to 100 different sets of color-coded polystyrene beads, each bearing a different assay. The Luminex reader is an essential tool that performs the key functions of this multiplex technology:

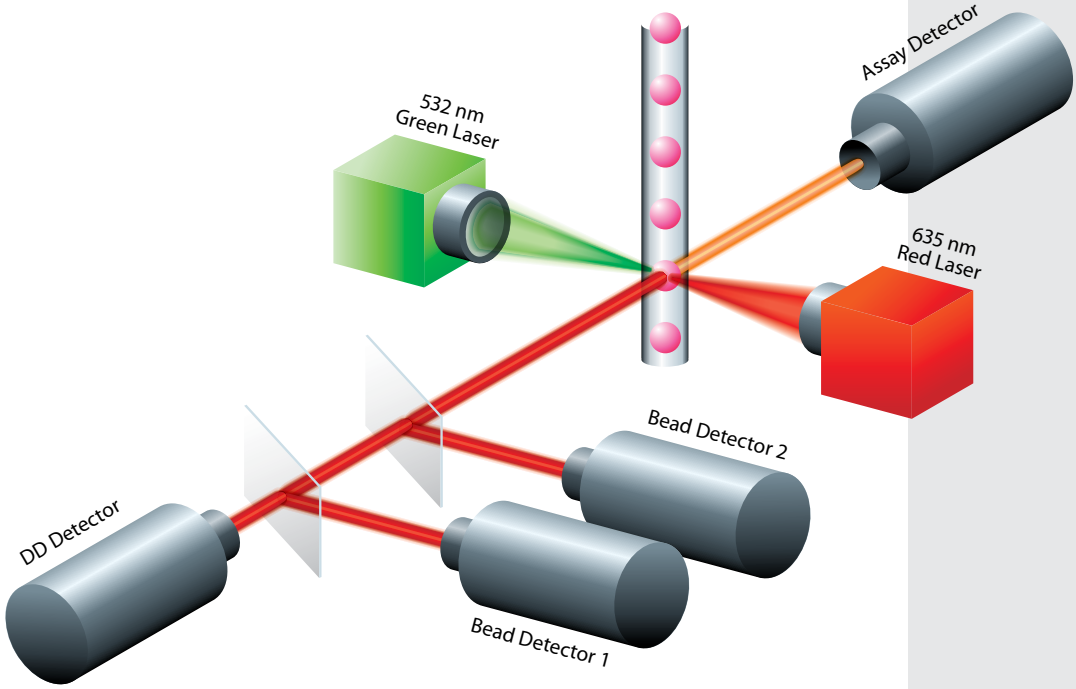


Fluidics—The reader detects individual beads by flow cytometry. The fluidics system of the reader aligns the beads into single file as they enter a stream of sheath fluid and then enter a flow cell. Once the beads are in single file within the flow cell, each bead is individually interrogated for bead color (analyte) and assay signal strength (PE fluorescence intensity)



Lasers—The reader uses a 532 nm green laser ("assay" laser) is used to excite the PE dye of the assay (Streptavidin-PE). The 635 nm solid state laser (red "classify" laser) is used to excite the dyes inside the beads to determine their "color" or "region" and is also used for doublet discrimination by light scatter

Detectors—The reader has four detectors, one for each of the optical paths shown in the figure below. Detectors are used to measure the fluorescence of the assay, to make bead determination (1-100) and the last to discriminate between single and aggregate beads.



Luminex Performance Highlights

Reduced cost and labor by multiplexing

Shortened time-to-results by favorable reaction kinetics of liquid bead array approach, with smaller sample requirements

Liquid reaction kinetics give faster, more reproducible results than with solid, planar arrays

Focused, flexible multiplexing in the range of 1 to 100 analytes meets the needs of a wide variety of applications—protein expression profiling, focused gene expression profiling

Panomics

For pricing and more information visit our website at
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