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 phospho-tyrosine 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 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**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of Detection</td>
<td>( \leq 2,000 ) transcripts/assay well</td>
</tr>
<tr>
<td>Limit of Quantitation</td>
<td>( \leq 5,000 ) transcripts/assay well</td>
</tr>
<tr>
<td>Linear Dynamic Range</td>
<td>( \geq 3 ) logs</td>
</tr>
<tr>
<td>Assay CV</td>
<td>( \leq 15% ) intra-assay; ( \leq 20% ) inter-assay</td>
</tr>
<tr>
<td>Compatible Sample Types</td>
<td>Cultured cells, whole blood, PAXgene blood or dried blood spots, fresh/frozen tissues, FFPE samples, purified RNA</td>
</tr>
<tr>
<td>Assay Format</td>
<td>96-well plate</td>
</tr>
<tr>
<td>Targets/well</td>
<td>3–30</td>
</tr>
</tbody>
</table>

**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

QuantiGene Plex 2.0

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

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.

**Step 1:** Incubation of the cis element probes with the nuclear extract or whole cell lysate in 96 well plate

**Step 2:** Transfer probe TF mixture to separation plate and wash TF bound probe mix. Denature cis elements away from TFs

**Step 3:** Denature cis elements by heat and aneal to Luminex beads with anti-sense sequence

**Step 4:** Add streptavidin PE to sample and read on Luminex machine
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.

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)

<table>
<thead>
<tr>
<th>RUNX/AML</th>
<th>ELK-1</th>
<th>ISRE</th>
<th>OCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP-1</td>
<td>ER</td>
<td>MEF-2</td>
<td>p53</td>
</tr>
<tr>
<td>AP-2</td>
<td>ETS/PEA</td>
<td>MYOD</td>
<td>PAX-3</td>
</tr>
<tr>
<td>AR</td>
<td>FAST-1</td>
<td>NF-1</td>
<td>PAX-5</td>
</tr>
<tr>
<td>ATF-2</td>
<td>FKH-1</td>
<td>NFAT</td>
<td>PPAR</td>
</tr>
<tr>
<td>BRN-3</td>
<td>GATA-1</td>
<td>NF-E1/YY1</td>
<td>SMAD</td>
</tr>
<tr>
<td>CEBP</td>
<td>GR/PR</td>
<td>NF-E2</td>
<td>STAT-1</td>
</tr>
<tr>
<td>C-MYB</td>
<td>HIF-1</td>
<td>NFkB</td>
<td>STAT-3</td>
</tr>
<tr>
<td>CREB</td>
<td>HNF1</td>
<td>NKB-2.5</td>
<td>STAT-4</td>
</tr>
<tr>
<td>E2F-1</td>
<td>IRF-1</td>
<td>NF-Y</td>
<td>STAT-5</td>
</tr>
</tbody>
</table>

Visit our website to see the most current list.

Procarta TF Panel 2 (43 TFs)

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

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

Add detection antibody and incubate for 30 minutes.

Add SAPE and incubate for 30 minutes.

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.

Simultaneous analysis of protein and gene expression from the same sample. Human histiocytic 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**

- L1-alpha
- L1-beta
- IL-2
- IL-4
- IL-5
- IL-6
- IL-7
- IL-8
- IL-10
- IL-12(p70)
- IL-12(p40)
- IL-13
- IL-17
- ENA-78
- EOTAXIN
- FGF-basic
- G-CSF

**Mouse 23**

- L1-alpha
- IL-1-beta
- IL-2
- IL-3
- IL-4
- IL-5
- IL-6
- IL-10
- IL-12(p40)
- IL-13
- IL-17
- PDGF-BB
- RANTES
- TGF-beta
- TGF-alpha
- VEGF

**Rat 9**

- L1-alpha
- IL-1-beta
- IL-6
- I-CAM
- KC
- MCP-1
- MIP-1-alpha
- RANTES
- TNF-alpha
- VEGF

**Specifications**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>1 pg/mL/cytokine</td>
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<tr>
<td><strong>Precision</strong></td>
<td></td>
</tr>
<tr>
<td>Average Inter-assay CV</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>Average Intra-assay CV</td>
<td>&lt;10%</td>
</tr>
<tr>
<td><strong>Spike Recovery</strong></td>
<td>80-120%</td>
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<tr>
<td><strong>Cross Reactivity</strong></td>
<td>Negligible</td>
</tr>
<tr>
<td><strong>Matrices</strong></td>
<td>Cell culture supernatants, serum, plasma</td>
</tr>
</tbody>
</table>
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.

Key Applications

- SH2 Profiling
- Peptide affinity screening
- Drug binding screening
- Phosphoprotein detection using specific antibody

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

Procarta SH2 Domain Plex

<table>
<thead>
<tr>
<th>SH2 Domain</th>
<th>Name</th>
<th>Bead Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>3BP2</td>
<td>CSK</td>
<td>P85B-D1</td>
</tr>
<tr>
<td>ABL2</td>
<td>VAV3</td>
<td>P85B-D2</td>
</tr>
<tr>
<td>BTK</td>
<td>LCK</td>
<td>PLCG1-D1</td>
</tr>
<tr>
<td>GRAP</td>
<td>LCP2</td>
<td>PTPN11-D2</td>
</tr>
<tr>
<td>CRK</td>
<td>MATK</td>
<td>PTPN6-D2</td>
</tr>
<tr>
<td>CRKL</td>
<td>NSP1</td>
<td>SOCS2</td>
</tr>
<tr>
<td>DAPP1</td>
<td>GRB2</td>
<td>STAP2</td>
</tr>
<tr>
<td>FYN</td>
<td>P55G-D1</td>
<td>SYK-D2</td>
</tr>
<tr>
<td>GRB10</td>
<td>P85A-D1</td>
<td>TNS</td>
</tr>
<tr>
<td>GRB14</td>
<td>P85A-D2</td>
<td>SHC1</td>
</tr>
</tbody>
</table>

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