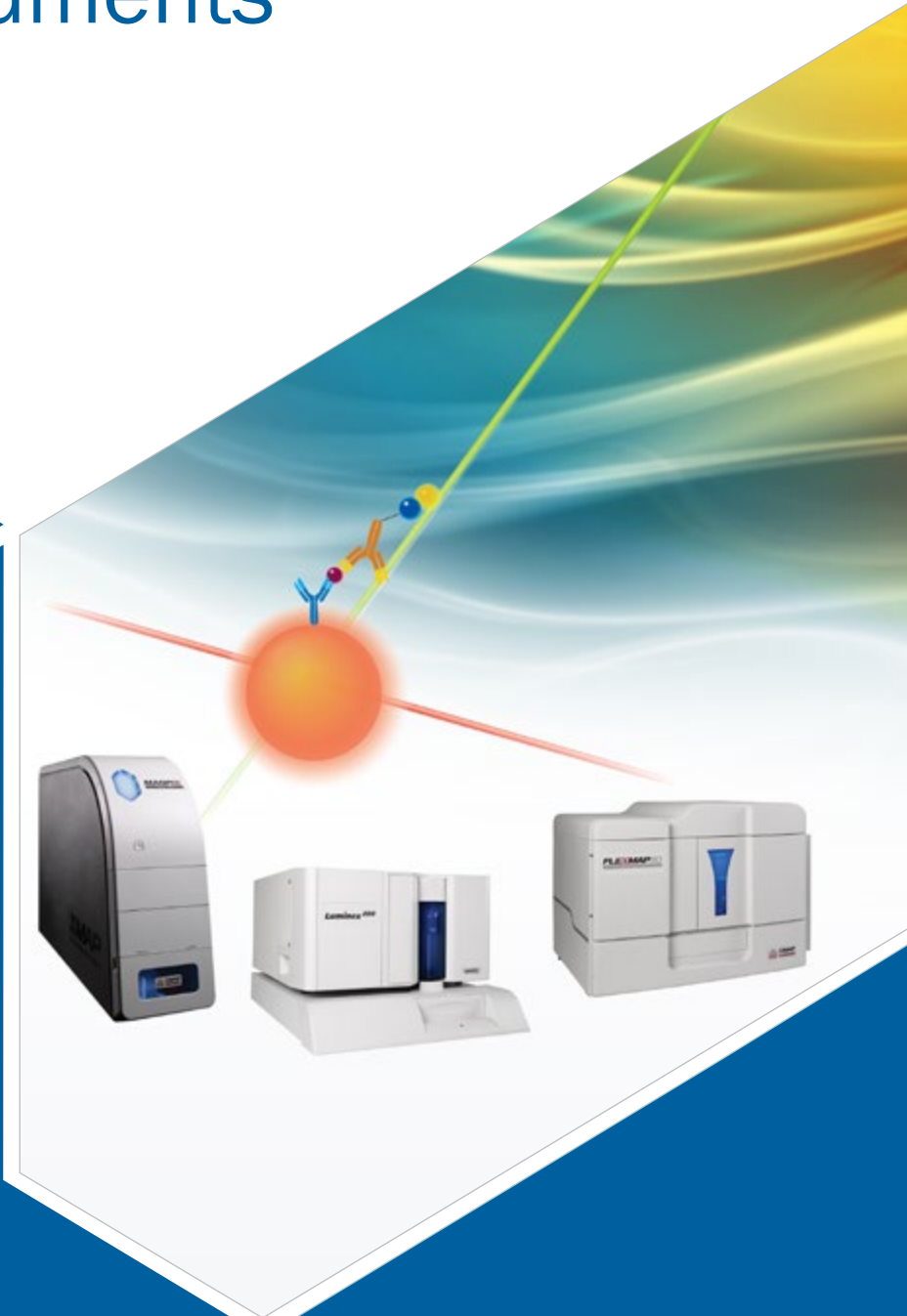


**R&D SYSTEMS**  
a **biotechne** brand

# Luminex<sup>®</sup> Assays and Instruments



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# The Benefits of Multiplexing

Examining multiple factors simultaneously in a single sample volume, also known as multiplexing, can provide numerous benefits to the user.

- **Maximizes Limited Sample:** Multiplexing allows the user to maximize data collection from a small sample volume.
- **Minimizes Experimental Variability:** Examining multiple factors at one time removes a layer of variability from data, as the sample is processed only once and multiple data points are derived from a single manipulation.
- **Optimizes Productivity:** Allowing users to collect multiple data points while minimizing sample preparation and processing saves time and generates high volumes of data.

## R&D Systems® Luminex® Bead-Based Assays for Multiplexing

R&D Systems offers three bead-based multiplex immunoassay formats utilizing Luminex xMAP® microparticle technology allowing users to better tailor assay selection to their individual research needs. R&D Systems Luminex assays are specifically designed to optimize the benefits and overcome the challenges of multiplexing.

### R&D Systems Luminex Assays

Luminex Assays from R&D Systems are designed to maximize multiplexing capacity and flexibility while maintaining assay specificity. Learn more on page 15.

- **Largest Luminex Multiplex Available:** simultaneously analyze up to 100 analytes
- **Flexible Analyte Selection:** choose from over 300 analytes
- **Unique Analytes Offered:** over 75 analytes are exclusively available from R&D Systems
- **Rapidly Expanding Menu:** new analytes are released monthly
- **Polystyrene or Magnetic Options:** all analytes are available in either the polystyrene or magnetic microparticle format

### R&D Systems Luminex High Performance Assays

Luminex High Performance Assays from R&D Systems are designed to maximize assay accuracy and precision while preserving the benefits of multiplexing. Learn more on page 19.

- **Accurate and Reproducible Results:** panel development and validation testing are similar to R&D Systems gold-standard Quantikine® ELISA assays
- **Polystyrene or Magnetic Options:** select panels are available in either the polystyrene or magnetic microparticle format
- **User-Defined Analyte Selection:** choose analytes from established panels and select “premixed” or “end-user mixed” options

### R&D Systems Luminex Immunoglobulin Isotyping Assays

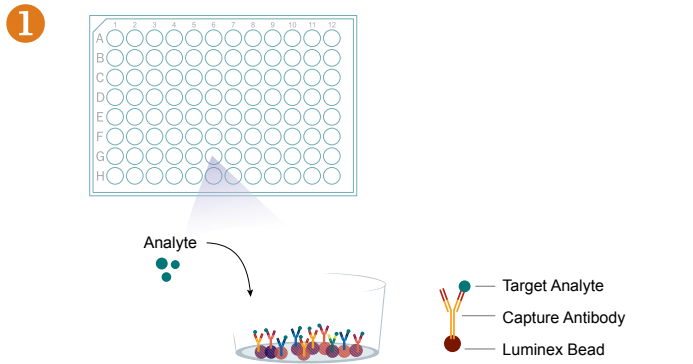
Luminex Immunoglobulin Isotyping Assays allow for the simultaneous quantitation of up to six immunoglobulin isotypes. Learn more on page 22.

- **Optimized:** All analytes are optimized and tested together for detection of their given analytes in a multiplex setting to ensure the best results possible
- **Flexible:** Measure only what you want! Select one or more of the analytes in a given panel
- **Rapid:** Get more done with results as fast as 1.5 hours
- **Choice:** Polystyrene or magnetic bead formats
- **Options:** Measure total immunoglobulin or investigate the kappa to lambda light chain ratio

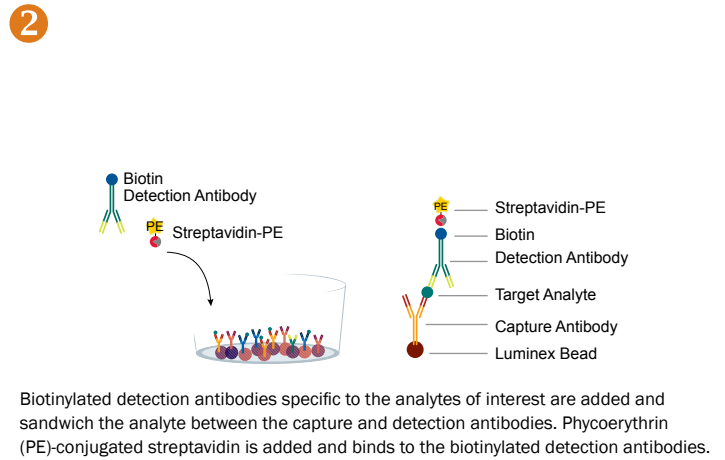
# R&D Systems® Luminex® Immunoassay Principle

## Principle Overview

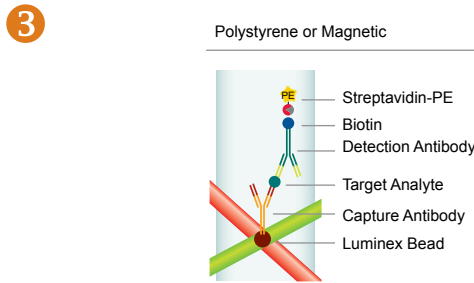
Luminex assays from R&D Systems are bead-based multianalyte profiling kits for detecting protein analytes in biological fluids. The kits utilize color-coded polystyrene or superparamagnetic microparticles coated with antibodies that recognize the specific target analytes. The different analyte-specific beads are mixed and incubated with the sample. Captured analytes are subsequently detected using a cocktail of biotinylated detection antibodies specific to each analyte and a streptavidin-phycoerythrin conjugate.



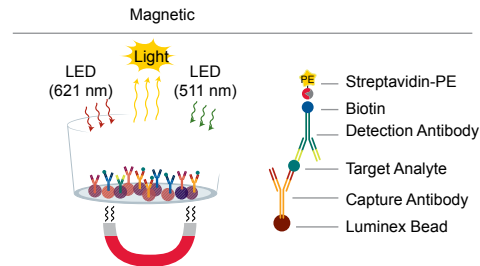
The sample is added to a mixture of color-coded beads, pre-coated with analyte-specific capture antibodies. The antibodies bind to the analytes of interest.



Biotinylated detection antibodies specific to the analytes of interest are added and sandwich the analyte between the capture and detection antibodies. Phycoerythrin (PE)-conjugated streptavidin is added and binds to the biotinylated detection antibodies.



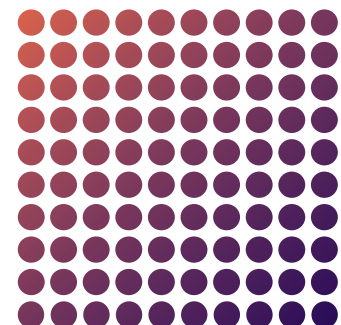
Polystyrene or magnetic beads are read on a dual-laser flow-based detection instrument, such as the Luminex 100/200™, Luminex FLEXMAP 3D®, or the equivalent Bio-Rad® BioPlex® analyzers. One laser classifies the bead and identifies the analyte being detected. The second laser determines the magnitude of the PE-derived signal, which is in direct proportion to the amount of analyte bound.



In addition to the Luminex 100/200, Luminex FLEXMAP 3D, or the equivalent Bio-Rad Bio-Plex analyzers, magnetic beads can also be read using the Luminex or Bio-Rad MAGPIX® Analyzer. A magnet in the MAGPIX analyzer captures and holds the magnetic beads in a monolayer, while two spectrally distinct light-emitting diodes (LEDs) illuminate the beads. One LED identifies the analyte that is being detected, and the second LED determines the magnitude of the PE-derived signal. A CCD camera captures images of each well.

## Luminex® xMAP® Microparticle Technology

xMAP technology by Luminex utilizes color-coded microparticles that incorporate different proportions of two dyes. The dyes can be excited by a laser at a single wavelength to provide a distinct spectral signature to each of 100 bead types or “regions.” This technology allows multiplexing of up to 100 assays simultaneously.



# Unique Multiplex Optimization Challenges

The simultaneous detection of multiple protein analytes presents many challenges. This includes assay interference and the need for optimized, yet multipurpose diluents. R&D Systems recognizes these unique difficulties. The diluents and antibody pairs used in our Luminex Assays are carefully developed and tested to ensure optimal analyte detection.

## Multipurpose Diluents

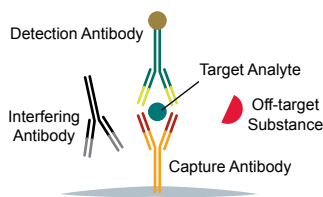
**Diluents used in multiplex assays may not be optimized for each analyte to the same degree that a diluent selected for a single analyte is.**

The most favorable conditions for promoting specific antibody/analyte binding and minimizing interference from other components will vary for each antibody/analyte set and for each sample type. Diluents provided with R&D Systems Luminex High Performance Assays have been tested and specifically formulated for the select analytes within each panel. The Luminex Assays provide diluents formulated for the widest possible variety of analyte combinations.

## Interference, Non-specific Binding, and Cross-reactivity

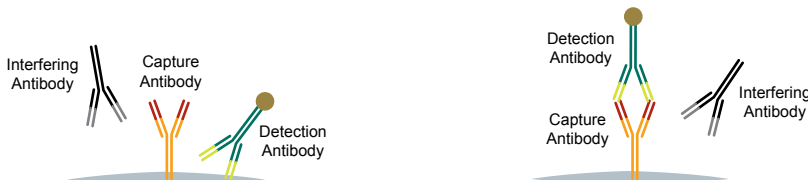
Reagents provided in R&D Systems Luminex Assays are carefully designed and tested to **minimize assay interference and ensure an optimally functional sandwich immunoassay** (Figure 1). Immunoassay interference occurs when a substance within the assay prevents the accurate detection of the target analyte. Immunoassay interference can occur due to non-specific antibody binding, antibody cross-reactivity, or antibody interference. In addition, complex sample matrices, such as serum and plasma, can often contain interfering factors. Interfering factors may also be introduced to biological samples via the reagents or equipment used. **Interference, no matter the mechanism, can result in either reduced or elevated signal relative to the actual concentration of target analyte.**

**Figure 1.** Optimally Functional Sandwich Immunoassay



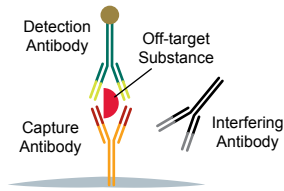
**Non-specific binding** occurs in an immunoassay when the antibody pair interacts with the sample container or other assay surfaces and contributes to background (Figure 2). Non-specific binding to microparticles, microplates, or other assay surfaces can be reduced by blocking. Diluents provided with R&D Systems Luminex Assays contain assay-optimized blocking reagents to ensure low non-specific binding.

**Figure 2.** Examples of Non-Specific Binding in an Immunoassay



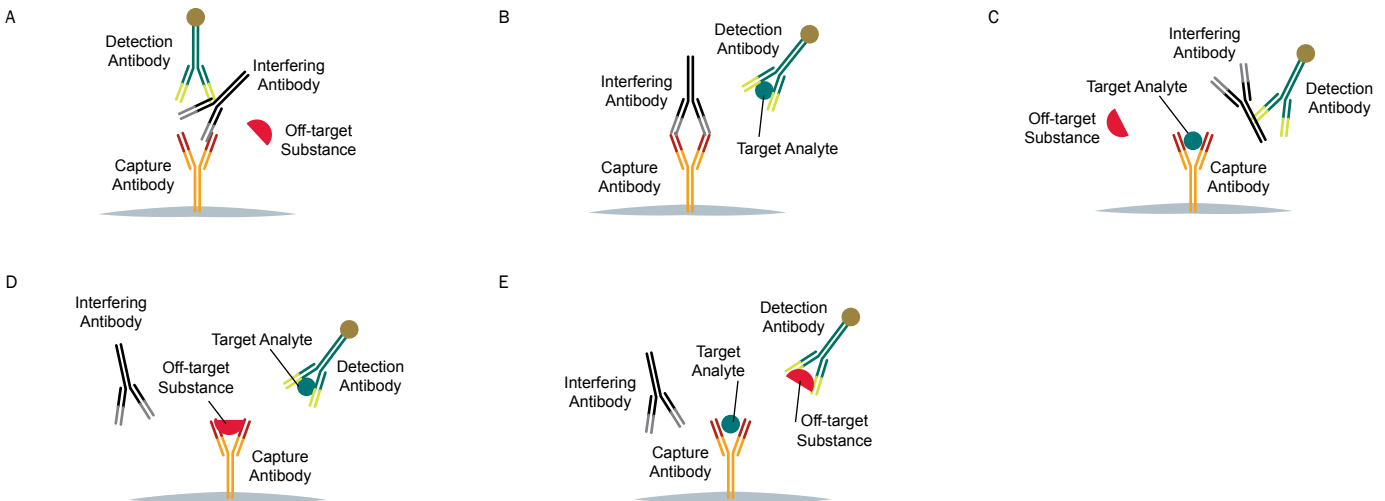
**Cross-reactivity**, the interaction of the antibody pair with a molecule other than the targeted analyte, can be caused by either the capture antibody or the detection antibody and often occurs when proteins in the sample are structurally similar to the analyte of interest (Figure 3). Optimizing antibodies so as to avoid cross-reactivity with substances within the biological sample is often challenging. R&D Systems carefully selects and exhaustively tests our in-house developed antibody pairs used in the Screening and Performance Luminex assays to ensure analyte specificity (Figure 7 on page 17).

**Figure 3.** Cross-Reactivity in an Immunoassay



**Antibody interference** occurs when endogenous antibodies within samples cross-link with assay antibodies (Figure 4; A-C) or substances within the sample (Figure 4; D, E) and prevent proper target-analyte binding to both the capture and detection antibodies. Common antibody-interfering substances in biological samples include human anti-mouse antibodies (HAMA) and rheumatoid factor (RF) antibodies as well as any substance present in exceptionally high concentrations. Diluents provided with R&D Systems Luminex Assays are designed to prevent antibody interference from HAMA and RF in biological samples (See Table 1 and 2).

**Figure 4.** Examples of Antibody Interference in an Immunoassay



**Table 1.** Common Interfering Substances Lead to False Positives in Competitor Luminex Assays

Normal					
Analyte	R&D Systems Luminex High Performance Assay	R&D Systems Luminex Assay	Competitor 1	Competitor 2	Competitor 3
TNF- $\alpha$	—	—	—	—	—
IFN- $\gamma$	—	—	—	—	—
GM-CSF	—	—	—	—	—
IL-10	—	—	—	+	—
IL-2	—	—	—	—	—
IL-4	—	—	—	—	—
IL-5	—	—	—	—	—
HAMA-high					
Analyte	R&D Systems Luminex High Performance Assay	R&D Systems Luminex Assay	Competitor 1	Competitor 2	Competitor 3
TNF- $\alpha$	—	—	—	—	—
IFN- $\gamma$	—	—	—	++	—
GM-CSF	—	—	—	+++	—
IL-10	—	—	—	++	—
IL-2	—	—	—	++	—
IL-4	—	—	—	—	—
IL-5	—	—	—	—	—
RF-high					
Analyte	R&D Systems Luminex High Performance Assay	R&D Systems Luminex Assay	Competitor 1	Competitor 2	Competitor 3
TNF- $\alpha$	—	—	—	++	+
IFN- $\gamma$	—	—	—	++	—
GM-CSF	—	—	—	+++	—
IL-10	—	—	—	+	—
IL-2	—	—	—	++	—
IL-4	—	—	—	++	+
IL-5	—	—	—	+	—

Serum samples confirmed to be Human Anti-Mouse Antibody-high (13-41.5 ng/mL; HAMA-high) or Rheumatoid Factor-high (30-42 IU/mL; RF-high) were purchased from ProMedDx. Normal serum was collected from apparently healthy donors. Seven analytes common to Luminex panels from R&D Systems and leading companies were run simultaneously. High concentrations of HAMA or RF caused false positive readings (i.e., measurable signal within the standard curve; +) by Luminex assays manufactured by two of the three competitor assays. No false positives were observed using the R&D Systems assays.

**Table 2.** Eliminate False Positives with R&D Systems Luminex Assays

Analyte	Sample	R&D Systems Mouse Luminex Assay*	Mouse Assay, Competitor 1*	Mouse Assay, Competitor 2*
GM-CSF	Human RF-High Sample 1	**	240	**
	Human RF-High Sample 2	**	367	**
IFN- $\gamma$	Human RF-High Sample 1	**	**	**
	Human RF-High Sample 2	**	**	204
IL-1 $\beta$	Human RF-High Sample 1	**	363	747
	Human RF-High Sample 2	**	636	1204
IL-10	Human RF-High Sample 1	**	**	420
	Human RF-High Sample 2	**	**	1382
TNF- $\alpha$	Human RF-High Sample 1	**	295	84
	Human RF-High Sample 2	**	2559	247

**R&D Systems Luminex Assays outperform the competition.** Human serum samples confirmed to be Rheumatoid Factor (RF)-high were purchased from BioreclamationIVT. Mouse Th1 cell analytes common to R&D Systems Luminex panels and two major competitors were tested simultaneously. The mouse analytes selected have no reported cross-reactivity with their respective human analytes. More than half of the samples tested using competitor assays yielded false positive results (measurable signal within the standard curve) due to the high concentrations of RF in the human samples. In contrast, no false positives were observed using the R&D Systems assay.

\*Results are pg/mL \*\*Below the lowest standard

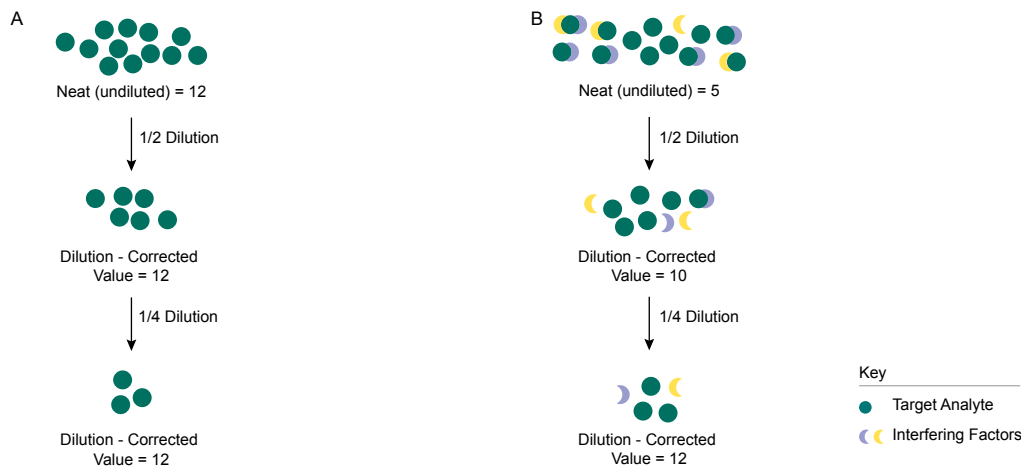
# Techniques to Evaluate Multiplex Accuracy

Interfering factors in an assay can result in the inaccurate measurement of the target analyte. Erroneous results in the form of artificially elevated or reduced analyte values can lead to incorrect experimental conclusions, which can lead to inappropriate subsequent experiments or the formation of flawed hypotheses. Assay validation methods, including linearity of dilution and recovery, can help to detect immunoassay interference and ensure accurate analyte measurement. R&D Systems tests linearity of dilution for Luminex Screening and Performance Assays for all validated sample types and performs spike recovery analysis for all Luminex High Performance Assays to ensure accurate analyte measurement.

## Sample Linearity of Dilution

R&D Systems Luminex Screening and Performance Assays are tested for assay linearity on validated sample types, and results indicate the accuracy of the determined analyte concentration. The principle of assay linearity maintains that after corrections for the dilution factor, sample dilutions should always derive the same final analyte concentration for a sample. **Interfering factors present in the sample matrix or the diluent can compromise assay linearity to varying degrees depending upon the magnitude of dilution** (Figure 5). Performing linearity of dilution curves can determine if interfering substances are present within biological samples and ensure that the final analyte concentrations detected are an accurate representation of the actual analyte concentration, regardless of the dilution factor. Additionally, as samples often need to be diluted to varying degrees so that the analyte of interest falls in the dynamic range of the standard curve, sample linearity of dilution ensures that the assay will allow for the comparison of sample values generated from samples run at different dilutions. In sample linearity of dilution experiments, serially diluted samples are assayed to determine if the expected concentrations of analyte are detected in dilution-corrected samples. Assay interference due to common interfering factors such as HAMA and RF can be detected using linearity of dilution in under-optimized immunoassays (Table 3).

**Figure 5.** Interfering Factors can Affect Linearity of Dilution



A. The expected results from a linearity of dilution experiment when no interfering factors are present in the sample or diluent matrix. B. The potential results from a linearity of dilution experiment if interfering factors are present in the sample matrix or diluent. Factors in complex matrices can interfere with analyte detection. This effect may be revealed by unexpected linear dilution values.



**Table 3. R&D Luminex Assays Show Linearity of Dilution Similar to Competitor Assays**

Analyte	Sample Type	R&D Systems Luminex High Performance Assay	R&D Systems Luminex Assay	Competitor 1	Competitor 2	Competitor 3
TNF- $\alpha$	Normal	107	99	125	122	112
	HAMA-high	115	104	108	123	123
	RF-high	104	83	118	155	ND
IFN- $\gamma$	Normal	115	90	130	131	119
	HAMA-high	105	97	106	107	127
	RF-high	102	108	115	111	121
GM-CSF	Normal	94	90	91	125	104
	HAMA-high	96	96	102	125	125
	RF-high	97	101	98	120	118
IL-10	Normal	102	99	111	126	111
	HAMA-high	97	90	108	120	117
	RF-high	97	61	112	111	107
IL-2	Normal	93	92	116	111	92
	HAMA-high	96	93	116	108	105
	RF-high	96	72	122	106	102
IL-4	Normal	104	90	110	122	97
	HAMA-high	108	95	107	111	125
	RF-high	102	103	118	111	113
IL-5	Normal	115	98	120	114	116
	HAMA-high	104	86	114	101	125
	RF-high	100	91	107	95	113

Sera from normal, HAMA-high, or RF-high individuals were pooled and spiked with either recombinant protein standard or natural protein from cell culture supernatants. Samples were assayed at the recommended dilution and further serially diluted (1:2). Results are presented as average percent observed from expected. Values between 70–130% indicate good assay linearity. Results outside of that range are highlighted in blue.

## Recovery

Recovery experiments help determine whether the diluent was able to overcome any interfering factors in the sample matrix that would otherwise hinder the assay's ability to accurately assess the concentration of the target analyte in reference to the standard curve. To conduct recovery experiments, a known concentration of either recombinant or natural target analyte is added (spiked) to the sample. The spiked samples are assayed to determine whether the expected concentrations of analyte were detected (i.e., recovered) when compared to the known spike concentration. Recovery of added proteins in R&D Systems Luminex High Performance Assays is typically between 75–125% in each validated sample type; specific data are available in the product datasheet for each analyte.

# Luminex® Instrumentation

R&D Systems Luminex Assays are compatible with all Luminex instrumentation systems. These systems can be obtained from R&D Systems. Please contact a Bio-Techne sales representative for more information: [sales@bio-technne.com](mailto:sales@bio-technne.com).



	MAGPIX	Luminex 100/200	FLEXMAP 3D
<b>Software</b>	xPONENT	xPONENT	xPONENT
<b>Optics</b>	LED/CCD Camera	Lasers/APDs/PMTs	Lasers/APDs/PMTs
<b>Hardware</b>	Fluorescent Imager	Flow Cytometry based	Flow Cytometry based
<b>Bead Compatibility</b>	MagPlex MagPlex-TAG	MagPlex MicroPlex MagPlex-TAG SeroMAP LumAvidin	MagPlex MicroPlex MagPlex-TAG SeroMAP LumAvidin
<b>Multiplex Capacity</b>	50	100 (80 for MagPlex)	500
<b>Read Time</b>	~ 60 mins/96-well plate	~ 45 mins/96-well plate	~ 20 mins/96-well plate ~ 75 mins/384-well plate
<b>Applications</b>	Protein/Nucleic Acid	Protein/Nucleic Acid	Protein/Nucleic Acid
<b>IVD-cleared Assays</b>	Yes	Yes	Yes
<b>Dynamic Range</b>	3.5 logs	3.5 logs	4.5 logs
<b>Microtiter Plate</b>	96 well	96 well	96 well and 384 well
<b>Dimensions, including computer (Width x Depth x Height)</b>	Approx. 70cm x 60cm x 50cm	Approx. 115cm x 60cm x 50cm	Approx. 110cm x 62cm x 63cm
<b>Weight (Analyzer)</b>	17.5 kg (38.5 lbs)	49 kg (113 lbs)	77.1 kg (170 lbs)
<b>Warranty</b>	1 year	1 year	1 year

## MAGPIX®

MAGPIX is the simplest, most affordable, and most portable of Luminex's xMAP instruments. Its self-cleaning routines and magnetic bead compatibility (with MagPlex beads) make MAGPIX extremely easy to learn and use.

- **Multiplexing:** up to 50 analytes per sample volume
- **Sensitivity:** single-digit picogram levels of protein
- **Dynamic Range (Typical):** >3.5 logs
- **Read Time:** 96-well plate in ≤ 60 min (up to 4,800 tests/hour)
- **Daily Start-Up/Shut-Down:** ≤ 15 min
- **Foot Print:** 3 ft linear bench space



## Included with the MAGPIX

Part Number	MAGPIX-XPONENT 4.2
Description	MAGPIX xPONENT 4.2 System
Core Instrument	MAGPIX bead reader with: Sample probe-needle; Off plate reagent block; Waste bottle; Sample probe height adjustment kit; Access door tool; Documentation
Available Power Cords (pick one nation)	USA, Australia, Brazil, Denmark, Germany, Sweden, France, Belgium, Spain, Switzerland, Israel, UK, Italy, Japan, India, China, S. Africa, Argentina, Korea, Taiwan
Bar-code scanner	Optional, at no charge with purchase of new system
Heater Block (96-well plate)	Optional, at no charge with purchase of new system
Included Software	xPONENT 4.2 (3 seats), Allele-call module (3 seats) Remote Web Monitoring module (3 seats)
Optional Software (extra charges apply)	LIS integration module Automation interface module Basic Security Module 21 CFR Part 11 Module (supersedes Basic Security) Additional seats
Reagents Included	MPX-CAL-K25, qty 1 (separately shipped) MPX-PVER-K25, qty 1 (separately shipped) MagPix Drive Fluid, qty 2 (ships with MAGPIX)
PC with Win 7 32-bit, US English	PC, Desktop, USFF, WIN 7 with XPON 4.2 Dell Optiplex "Ultra Small Form Factor" Dell Warranty Transfer Stand, All-In-One Monitor/PC USFF Dell 19" LCD monitor Dell sound-bar Dell Keyboard & mouse



## MAGPIX Reagents

The MAGPIX instrument requires drive fluid to serve as the delivery medium to transport the beads to the instrument's optics. Due to the compact nature of the MAGPIX system, drive fluid is available in small pre-filled disposable containers which are stored within the instrument. Each drive fluid container provides enough fluid to read approximately ten plates of samples, and they are sold in packages of four. MAGPIX uses a calibration kit and a verification kit, each providing 25 uses.

## Luminex® 200™

The Luminex 200 sets the standard for multiplexing. The ability to use either magnetic or polystyrene beads makes this instrument a popular choice for laboratories needing greater flexibility.

- **Multiplexing:** Up to 100 analytes per sample volume (80 on MagPlex microspheres)
- **Dynamic Range (Typical):** >3.5 logs
- **Read Time:** 96-well plate in ≤ 45 min (up to 12,800 tests/hour)
- **Daily Start-Up/Shut-Down:** ≤ 30 min



### Included with the Luminex 200

Part Number	LX200-XPON31
<b>Description</b>	Luminex 200 System with xPONENT 3.1
<b>Core Instrument</b>	Luminex 200 flow-based bead reader with: Sheath Delivery System; X-Y Platform for 96-well plates; Sample probe-needle; 1L sheath bottle, 1.2L Waste bottle; Sample probe height adjustment kit; Automated Maintenance Plate; Heater Block (96 well plate); Documentation
<b>Available Power Cords (pick one nation)</b>	USA, Australia, Brazil, Denmark, Germany, Sweden, France, Belgium, Spain, Switzerland, Israel, UK, Italy, Japan, India, China, S. Africa, Argentina, Korea, Taiwan
<b>Bar-code scanner</b>	Optional, at no charge with purchase of new system
<b>Included Software</b>	xPONENT 3.1 (3 seats) Allele-call module (3 seats) Remote Web Monitoring module (3 seats)
<b>Optional Software (extra charges apply)</b>	LIS integration module Automation interface module Basic Security Module 21 CFR Part 11 Module (supersedes Basic Security) Additional seats
<b>Reagents Included</b>	LX200-CAL-K25, qty 1 (separately shipped) LX200-CON-K25, qty 1 (separately shipped) xMAP Sheath Fluid (20L), qty 1 (ships with LX200)
<b>Desktop PC with Win 7 32-bit, US English &amp; xPONENT 3.1</b>	Dell Optiplex with 2 Port PCI RS232 Serial Adapter Card Dell Warranty Transfer Dell 17" LCD monitor with soundbar Dell Keyboard & mouse



### Luminex 100/200 Reagents

The Luminex 100 and Luminex 200 instruments require sheath fluid to serve as the delivery medium to transport the beads to the instrument's optics. Both instruments have the option of a 20L disposable external sheath fluid container or a compact 20X sheath fluid concentrate pack; which can be reconstituted, with water, to a working volume of 20L when needed. The Luminex 100/200 instruments, operating on xPONENT software, are calibrated using a calibration and a verification kit, each providing 25 uses.

## FLEXMAP 3D®

The FLEXMAP 3D is Luminex's most advanced and versatile multiplexing platform. Fast read time, compatibility with 96- and 384-well plates, and ease of integration with front-end automation systems make the FLEXMAP 3D the platform of choice for high-throughput applications.



- **Multiplexing:** Up to 500 analytes in a single sample volume
- **Dynamic Range (Typical):** >4.5 logs
- **Read Time:** 96-well plate in ≤ 20 min (up to 144,000 tests/hour); 384-well plate in ≤ 75 min (up to 153,600 tests/hour)
- **Daily Start-Up/Shut-Down:** ≤ 30 min

### Included with the FLEXMAP 3D

Part Number	FLEXMAP-3D-RUO
<b>Description</b>	FLEXMAP 3D xPONENT 4.2 System
<b>Core Instrument</b>	FLEXMAP 3D flow based bead reader with: Sample probe-needle; Off plate reagent block; Waste container; Sample probe height adjustment tool; Barcode Scanner; Heater Block 96-well; Heater Block 386- well; Documentation
<b>Available Power Cords (pick one nation)</b>	USA, Australia, Brazil, Denmark, Germany, Sweden, France, Belgium, Spain, Switzerland, Israel, UK, Italy, Japan, India, China, S. Africa, Argentina, Korea, Taiwan, Pakistan
<b>Included Software</b>	xPONENT 4.2 (3 seats), Allele-call module (3 seats) Remote Web Monitoring module (3 seats)
<b>Optional Software (extra charges apply)</b>	LIS integration module Automation interface module Basic Security Module 21 CFR Part 11 Module (supersedes Basic Security) Additional seats
<b>Reagents Included</b>	F3D-CAL-K25, qty 1 (separately shipped) F3D-PVER-K25, qty 1 (separately shipped) Sheath Fluid (20L) qty 1 (Ships with FLEXMAP 3D)
<b>PC with Win 7 32-bit, US English</b>	PC, Desktop, USFF, WIN 7 with XPON 4.2 Dell OptiPlex 7010 "Ultra Small Form Factor" Dell Warranty Transfer Arm Mounted ELO 19" Touch Screen Monitor AX2010 USB Stereo Speaker System Keyboard & Mouse



### FLEXMAP 3D Reagents

The FLEXMAP 3D instrument requires sheath fluid to serve as the delivery medium to transport the beads to the instrument's optics. This instrument has the option of a 20L disposable external sheath fluid container or a compact 20X sheath fluid concentrate pack; which can be reconstituted, with water, to a working volume of 20L when needed. The FLEXMAP 3D instrument is calibrated using a calibration and a verification kit, each providing 25 uses.

## xMAP® Instrument Reagents

Instrument	Reagent	Pack Size	Part Number
<b>MAGPIX</b>	MAGPIX Drive Fluid, 4 x 750 mL containers MAGPIX Calibration Kit MAGPIX Performance Verification Kit	4 pack 25 uses 25 uses	MPXDF-4PK MPX-CAL-K25 MPX-PVER-K25
<b>Luminex 100/200 (all versions)</b>	Sheath Fluid Sheath Concentrate Pack (20x)	20 L 1L	40-50000 40-75680
<b>Luminex 100/200 (xPONENT only)</b>	Luminex 100/200 Calibration Kit Luminex 100/200 Performance Verification Kit	25 uses 25 uses	LX200-CAL-K25 LX200-CON-K25
<b>FLEXMAP 3D</b>	Sheath Fluid Sheath Concentrate Pack (20x) FLEXMAP 3D Calibration Kit FLEXMAP 3D Performance Verification Kit	20L 1L 25 uses 25 uses	40-50000 40-75680 F3D-CAL-K25 F3D-PVER-K25



### Magnetic Plate Separator

The Luminex Magnetic Plate Separator is a modified plate magnet intended to separate a 96-well plate of MagPlex Microspheres during assay preparation steps. The side clasps on this magnet have been specially designed to hold a microplate securely to the magnet, enabling the user to quickly invert and decant it over a waste container. Similar to the method used in an ELISA, this manual plate washing method has been shown to effectively remove nearly all of the supernatant from every well on the plate, with virtually no bead carry-over.

## Instrument Service Plans

	Luminex 200 & FLEXMAP 3D	MAGPIX Only
<b>Number of visits for emergency repair</b>	Unlimited	Unlimited
<b>Emergency repair costs included in plan</b>	All Costs Included	All Costs Included
<b>Preventative maintenance visits per year</b>	One	One
<b>On-site response time (business days)</b>	Two	Two
<b>Priority scheduling for service requests</b>	Included	Included
<b>Product updates and hardware modifications</b>	Included	Included
<b>Software support</b>	Included	Included
<b>Unlimited 24x7x365 remote support</b>	Included (Unlimited, during business hours - Japan)	Included
<b>On-site assay support, as needed (Luminex Assays Only)</b>	Not Included	Not Included

# Luminex® Assays

Luminex Assays offer our widest selection of analytes for bead-based multianalyte profiling using cell culture supernatants, serum, or plasma samples. Luminex Assays are ideal for researchers needing to evaluate multiple analytes for trends in protein expression in disease states or following treatment. (See data obtained with the Luminex Assay in Figure 8 on page 18.) They are also valuable for examining combinations of analytes not available in defined panels. Luminex Assays allow the simultaneous profiling of up to 100 biomarkers when using polystyrene microparticles or 50 biomarkers when using magnetic microparticles. The analytes for this user-defined multiplex assay are available from a rapidly expanding menu and are supplied as premixed kits.

## Features

- **Flexible:** Simultaneously profile up to 100 analytes of your choice when using polystyrene beads or up to 50 analytes when using magnetic beads.
- **Economical:** Cost-effectively assay multiple markers.
- **Versatile:** Choose from a rapidly expanding analyte menu, including many unique analytes.
- **Species Variety:** Find kits designed for human, mouse, and rat analytes.
- **Rapid:** Run a multiple analyte assay in 3–3.5 hours.
- **Efficient:** Assay multiple analytes in a small sample volume (< 50 µL).

## Validation and Testing

Luminex Assays are validated for use with cell culture supernatants, plasma, or serum. All Luminex Assays are tested for sensitivity, intra-assay precision, inter-assay precision, and to ensure assay linearity for validated sample types. Antibody pairs are selected and tested to confirm the parallel detection of natural and recombinant standard protein and to ensure the accurate determination of target analyte within biological samples (Figure 6). Assays for each target analyte are screened against all target analytes to confirm low antibody cross-reactivity (Figure 7 on page 17).

## Formats

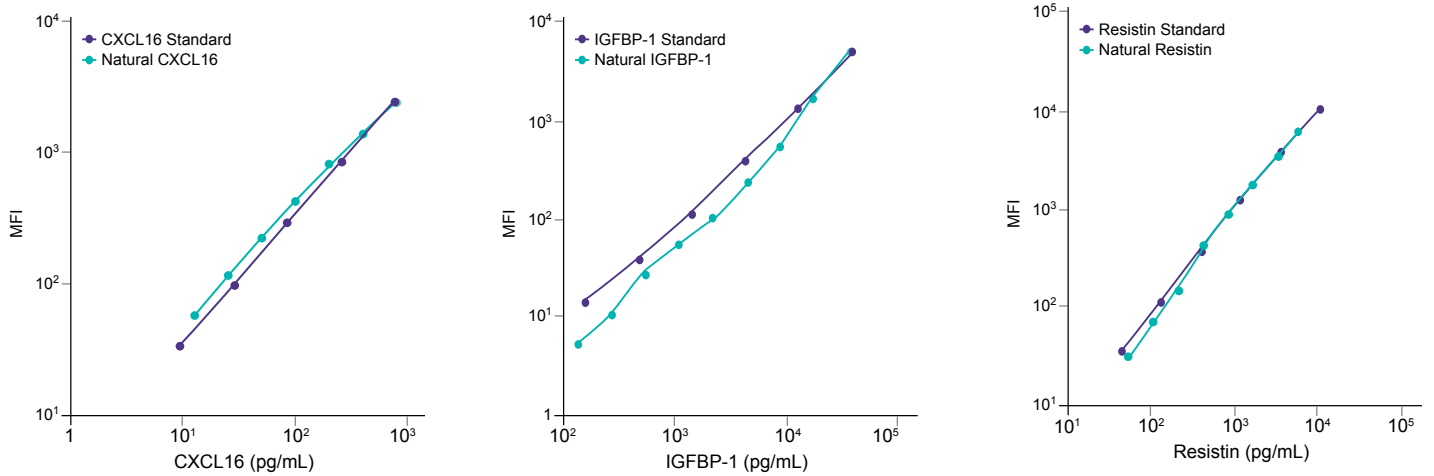
All analytes are available in polystyrene or magnetic bead formats.

- **Polystyrene beads:** Assays using polystyrene beads are compatible with the Luminex 100, Luminex 200, Luminex FLEXMAP 3D, Bio-Plex® 200, or Bio-Plex 3D analyzers.
- **Magnetic beads:** Assays using magnetic beads are compatible with the Luminex MAGPIX, Luminex 100, Luminex 200, Luminex FLEXMAP 3D, Bio-Plex MAGPIX, Bio-Plex 200, or Bio-Plex 3D analyzers.

## Kit Contents

Luminex Assay Kits contain a premixed cocktail of antibody-coated microparticles, premixed biotinylated detection antibodies, standard cocktail(s), microparticle diluent, biotin antibody diluent, standard/sample diluent, wash buffer, Streptavidin-PE, filter- or flat-bottom 96-well microplate, foil plate sealers, mixing bottles, and Certificate of Analysis.

**Figure 6.** Luminex Assays Accurately Detect Natural Proteins



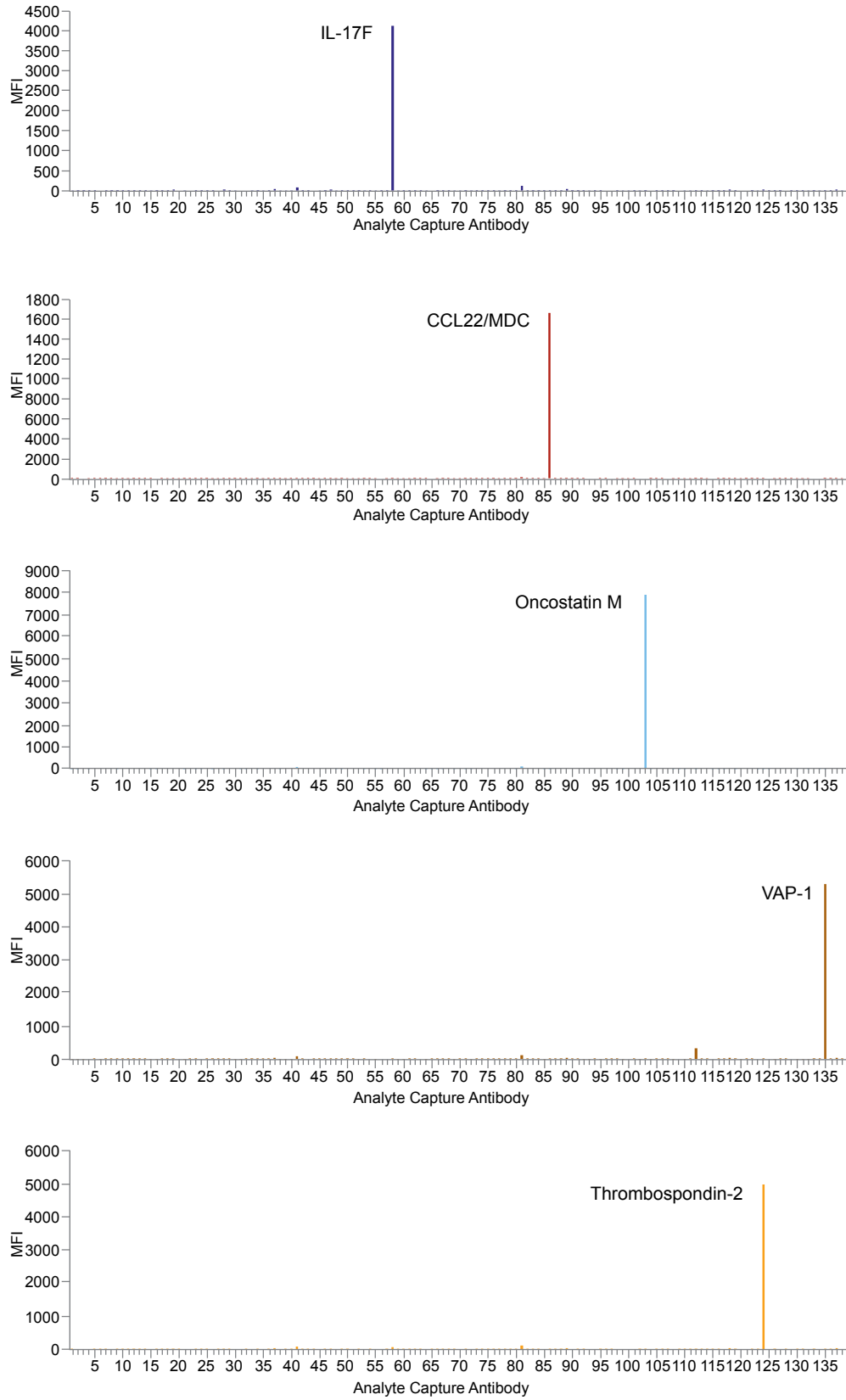
All Luminex Assays are tested to ensure that they detect natural proteins in parallel with detection of the recombinant protein standard. Representative dilution curves detecting natural or recombinant standard Resistin, CXCL16, and IGFBP-1 are shown. Parallel detection of natural and recombinant standard proteins ensures that the concentration of analyte within a biological sample can be accurately determined in comparison to the recombinant protein used to generate the standard curve.

**Key for Figure 7**

- |  |  |                               |   |
|--|--|-------------------------------|---|
| 1 ..... Adiponectin/Acrp30                   | 35 ..... FGF-21                            | 70 ..... IL-33                | 106 ..... CCL18/PARC                              |
| 2 ..... Aggrecan                             | 36 ..... FLRG                              | 71 ..... IL-34                | 107 ..... Proprotein Convertase 9/<br>..... PCSK9 |
| 3 ..... Angiopoietin-2                       | 37 ..... Follistatin                       | 72 ..... IL-4                 | 108 ..... Periostin/OSF-2                         |
| 4 ..... APP                                  | 38 ..... Galectin-3                        | 73 ..... IL-5                 | 109 ..... CXCL4/PF4                               |
| 5 ..... BAFF/BLyS/TNFSF13B                   | 39 ..... G-CSF                             | 74 ..... IL-6                 | 110 ..... Progranulin                             |
| 6 ..... BCMA                                 | 40 ..... GDF-15                            | 75 ..... IL-6 R               | 111 ..... RAGE                                    |
| 7 ..... CXCL13/BLC/BCA-1                     | 41 ..... GM-CSF                            | 76 ..... IL-8                 | 112 ..... TRANCE/RANK L/<br>..... TNFSF11         |
| 8 ..... BMP-2                                | 42 ..... CXCL1/Gro-α                       | 77 ..... IL-9                 | 113 ..... CCL5/RANTES                             |
| 9 ..... BMP-4                                | 43 ..... HB-EGF                            | 78 ..... CXCL10/IP-10         | 114 ..... Renin                                   |
| 10 ..... BMP-9                               | 44 ..... HGF                               | 79 ..... CXCL11/I-TAC         | 115 ..... Resistin                                |
| 11 ..... CD14                                | 45 ..... Human Growth<br>..... Hormone/hGH | 80 ..... Leptin               | 116 ..... ROBO4                                   |
| 12 ..... CD163                               | 46 ..... ICAM-1/CD54                       | 81 ..... CCL2/MCP-1           | 117 ..... SHBG                                    |
| 13 ..... CD27/TNFRSF7                        | 47 ..... IFN-γ                             | 82 ..... CCL8/MCP-2           | 118 ..... SOST                                    |
| 14 ..... CD30/TNFRSF8                        | 48 ..... CD40 Ligand/TNFSF5                | 83 ..... CCL7/MCP-3           | 119 ..... SPARC                                   |
| 15 ..... CD40 Ligand/TNFSF5                  | 49 ..... IGFBP-1                           | 84 ..... CCL13/MCP-4          | 120 ..... SP-D                                    |
| 16 ..... Chitinase 3-like 1/<br>..... CHI3L1 | 50 ..... IGFBP-3                           | 85 ..... M-CSF                | 121 ..... ST2/IL-1 R4                             |
| 17 ..... Cripto-1                            | 51 ..... IL-1ra/IL-1F3                     | 86 ..... CCL22/MDC            | 122 ..... TACI/TNFRSF13B                          |
| 18 ..... C-Reactive Protein/CRP              | 52 ..... IL-1 RI                           | 87 ..... MFG-E8               | 123 ..... CCL17/TARC                              |
| 19 ..... Cardiac Troponin I                  | 53 ..... IL-1 RII                          | 88 ..... CXCL9/MIG            | 124 ..... Thrombospondin-2                        |
| 20 ..... CXCL16                              | 54 ..... IL-10                             | 89 ..... CCL3/MIP-1α          | 125 ..... Tenascin C                              |
| 21 ..... DcR3/TNFRSF6B                       | 55 ..... IL-12 p70                         | 90 ..... CCL4/MIP-1β          | 126 ..... TFPI                                    |
| 22 ..... Dkk-1                               | 56 ..... IL-13                             | 91 ..... CCL20/MIP-3α         | 127 ..... Tie-1                                   |
| 23 ..... DR3/TNFRSF25                        | 57 ..... IL-17                             | 92 ..... MMP-1                | 128 ..... Tie-2                                   |
| 24 ..... TRAIL R2/DR5/<br>..... TNFRSF10B    | 58 ..... IL-17F                            | 93 ..... MMP-12               | 129 ..... TNF RI/TNFRSF1A                         |
| 25 ..... EGF                                 | 59 ..... IL-18 BPa                         | 94 ..... MMP-13               | 130 ..... TNF RII/TNFRSF1B                        |
| 26 ..... CXCL5/ENA-78                        | 60 ..... IL-19                             | 95 ..... MMP-2                | 131 ..... TNF-α                                   |
| 27 ..... ENPP-2/Autotaxin                    | 61 ..... IL-1α                             | 96 ..... MMP-3                | 132 ..... Thrombopoietin/Tpo                      |
| 28 ..... CCL26/Eotaxin-3                     | 62 ..... IL-1β                             | 97 ..... MMP-7                | 133 ..... TRAIL R3/TNFRSF10C                      |
| 29 ..... EphA2                               | 63 ..... IL-2                              | 98 ..... MMP-8                | 134 ..... Uteroglobin                             |
| 30 ..... E-Selectin/CD62E                    | 64 ..... IL-22                             | 99 ..... MMP-9                | 135 ..... VAP-1                                   |
| 31 ..... Complement Factor D                 | 65 ..... IL-23                             | 100 ..... Myeloperoxidase/MPO | 136 ..... VCAM-1                                  |
| 32 ..... Fas/TNFRSF6                         | 66 ..... IL-27                             | 101 ..... Cardiac Myoglobin   | 137 ..... VEGF                                    |
| 33 ..... Fas Ligand/TNFSF6                   | 67 ..... IL-28A                            | 102 ..... NRG1-β 1/HRG1-β 1   | 138 ..... VEGF R1                                 |
| 34 ..... FGF basic                           | 68 ..... IL-2 Rα                           | 103 ..... Oncostatin M/OSM    | 139 ..... VEGF R3                                 |
|  | 69 ..... IL-31                             | 104 ..... P-Selectin/CD62P    |   |
|  |  | 105 ..... Serpin E1/PAI-1     |   |



Figure 7. R&D Systems Luminex Assays are Analyte-Specific



Luminex Assay analytes, capture antibodies, and detection antibodies are individually tested to ensure minimal cross-reactivity between analytes and antibody pairs. Oncostatin M, CCL22/MDC, VAP-1, IL-17F, and Thrombospondin-2 detection antibodies were tested against 139 capture antibodies and proteins in sets of approximately 20. No cross-reactivity was observed between detection antibodies and off-target proteins.

**Figure 8.** R&D Systems Luminex Assay Used to Detect Proteins Secreted by Various Breast Cancer Cell Lines

	MCF-7	MDA-MB-231	SK-BR-3	T47D	ZR-75-1
IL-6	1.88	130	0	0	0
MMP-3	0	45.2	0	0	0
CXCL8/IL-8	6.95	1300	11.6	0	0
MMP-1	31.6	<HIGH>	4.71	0	0
MMP-2	551	1110	965	353	1700
CCL2/MCP-1	0	3.09	532	25.4	0
VEGF	729	1970	1420	466	4880
MMP-13	17.4	38.3	733	22.6	0
MMP-12	1310	1360	1130	1140	1320
CCL5/RANTES	25.5	0	0	0	0
IL-2	44.7	64.3	64.3	28.1	26.2
Adiponectin	1280	1620	1250	867	1180
FGF basic	29.6	32.4	26.1	24.3	29.6
G-CSF	0	40.1	0	0	0
GDF-15	547	0	91.7	10.4	139
Aggrecan	510	555	490	400	443
IL-23	1250	1220	1100	1140	1340
CXCL1/Gro $\alpha$	32	168	42	48.3	44.4
FGF-21	17800	19700	18400	17400	18100
CHI3-L1	145	367	289	131	301
EGF	66.3	66.1	59.1	73.7	64.9
Angiopoietin-2	300	480	260	235	180
Dkk-1	5080	1310	404	270	0
Galectin-3	1870	82.4	0	94.4	500
IL-22	224	323	302	283	324
CCL8/MCP-2	52.4	121	197	0	0
Cardiac Myoglobin	7.6	0	31.8	3.27	101
Serpin E1/PAI-1	2630	16200	46000	2.04	7.5
Proprotein Convertase 9	3820	3560	2970	2770	4140
DcR3	5300	4630	3780	4720	4310
TNF RI	525	163	144	237	130



The MCF-7, MDA-MB-231, SK-BR-3, T47D, and ZR-75-1 breast cancer cell lines were cultured under optimal cell line-specific culture conditions. Cell culture supernatants were collected and analyzed for the secretion of 99 protein analytes using a single Luminex Assay. Sixty-eight analytes were undetectable. Data are presented in pg/mL. Coloration indicates the sample concentration with regard to the standard curve for each analyte, which was generated using 1:3 serial dilution of the standards provided with each Luminex Assay. The standard curve ranged between 1:1-1:243 relates to the supplied protein standard.

# Luminex<sup>®</sup> High Performance Assays

Luminex High Performance Assays are our most accurate and precise bead-based multianalyte profiling kits. High Performance Assays are designed for researchers quantitating a defined set of analytes and needing the highest multiplex assay performance for select analytes with regard to recovery, linearity, and lot-to-lot reproducibility. In-house testing indicates that analyte concentrations determined with our Luminex High Performance Assays correlate closely with those obtained with our Quantikine ELISAs (Figure 9). Performance Assays are fully validated for each sample type listed. The assays rely on diluents optimized for each panel, providing maximum performance for measuring the specific analytes in that panel. Analyte-specific reagents are available either as a complete panel or as a smaller subset from any panel. The kits can be used to measure target analytes in their validated sample types, which can include cell culture supernatants, serum, plasma, urine, saliva, and milk (Figure 10 on page 21).

## Features

- **Optimized:** All analytes in a given panel are optimized and tested together to ensure maximum performance for the entire panel.
- **Validated:** Kits undergo validation testing similar to tests conducted on the Quantikine ELISA single analyte assays. The kits are correlated with Quantikine ELISAs, when available.
- **Flexible:** Order base kits and bead sets individually or as premixed kits.
- **Rapid:** Assay multiple analytes in 2.5–5 hours.
- **Efficient:** Measure multiple analytes in a small sample volume ( $\leq 100 \mu\text{L}$ ).

## Validation and Testing

Our Luminex High Performance Assays undergo our most extensive validation testing. All Luminex High Performance Assay Panels are validated for use with plasma and serum samples. Some High Performance Assays are additionally validated for cell culture supernatants, milk, saliva, or urine, as indicated on the individual product data sheets. Luminex High Performance Assays are tested for sensitivity (three-quarters the low standard), intra-assay precision, inter-assay precision, and to ensure assay linearity for validated sample types. Recovery values for individual samples in validated sample types are also tested. Validation data for each analyte can be found in the product datasheet.

## Formats

Analytes are available in polystyrene or magnetic bead formats.

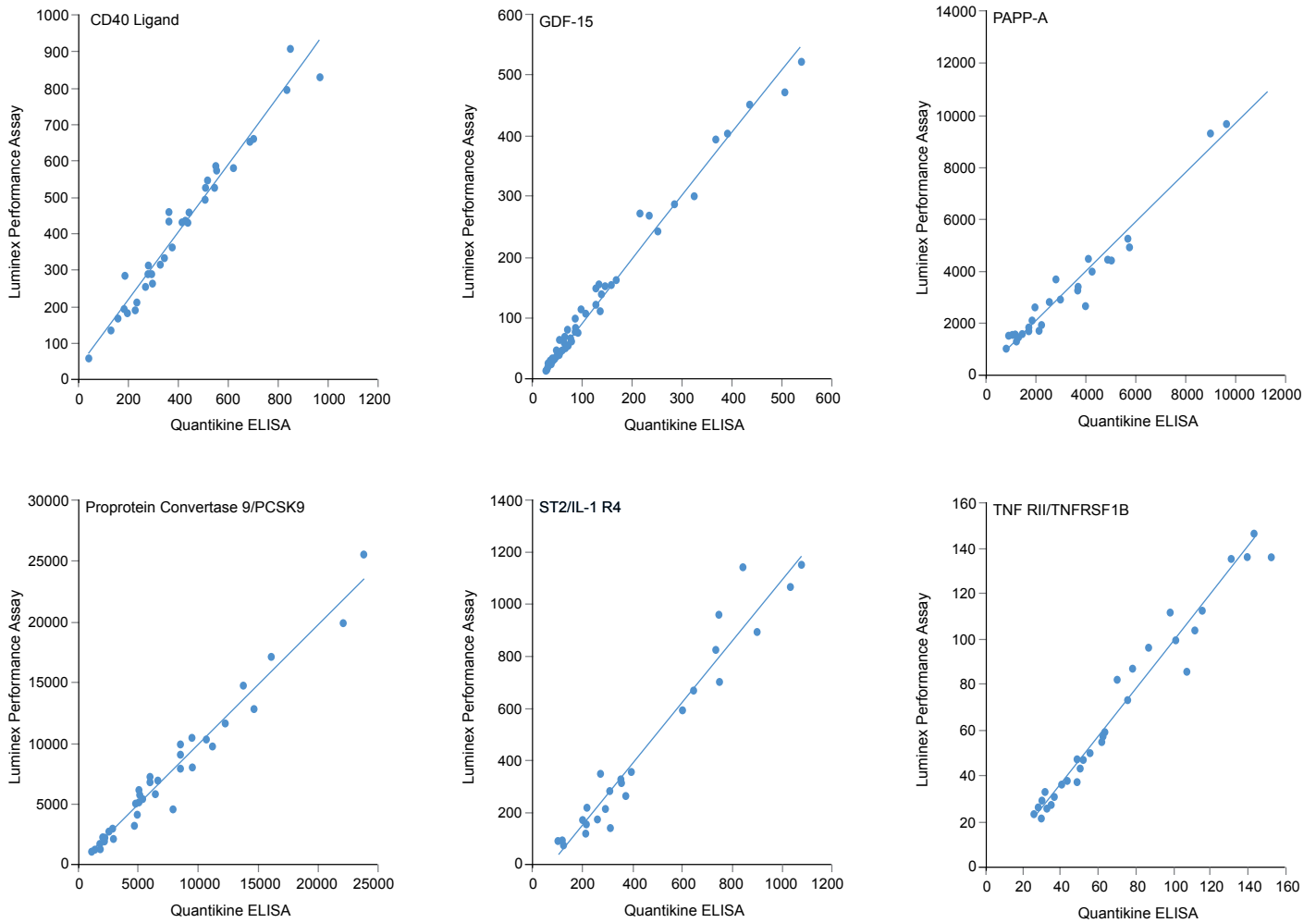
- **Polystyrene beads:** Assays using polystyrene beads are compatible with the Luminex 100, Luminex 200, Luminex FLEXMAP 3D, Bio-Plex 200, or Bio-Plex 3D analyzers.
- **Magnetic beads:** Assays using magnetic beads are compatible with the Luminex MAGPIX, Luminex 100, Luminex 200, Luminex FLEXMAP 3D, Bio-Plex MAGPIX, Bio-Plex 200, or Bio-Plex 3D analyzers.

## Kit Contents\*

- **Base Kits:** Standard cocktail(s), microparticle diluent, biotin antibody diluent, calibrator diluent(s), Streptavidin-PE, wash buffer concentrate, mixing bottles, filter- or flat-bottom microplate, foil plate sealers
- **Bead Sets:** Antibody-coated microparticles, biotinylated detection antibodies

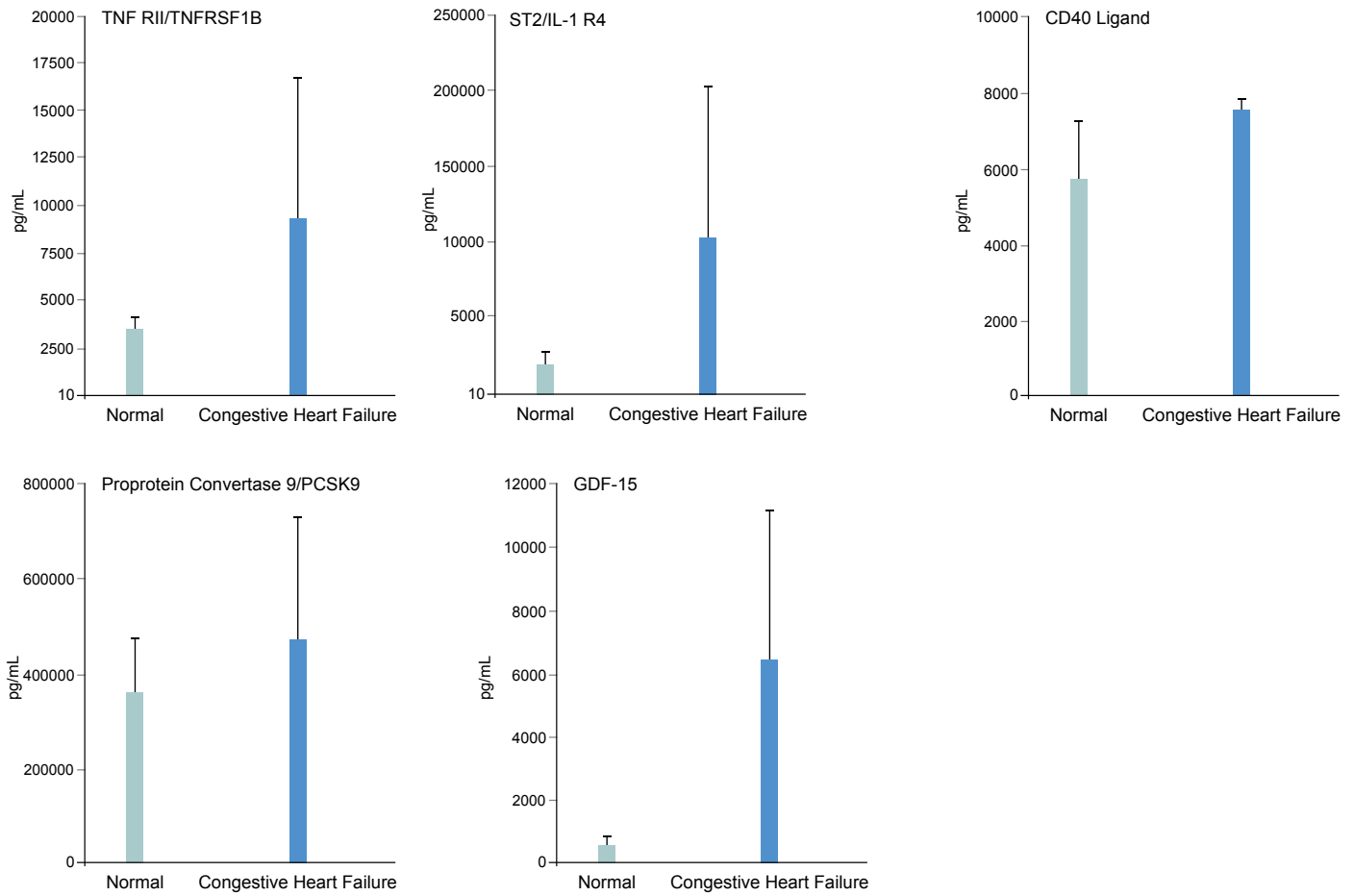
\*Base Kits and Bead Sets can be ordered individually or as premixed kits.

**Figure 9.** Analyte Concentrations Determined Using the Luminex Performance Cardiac Panel A are Consistent with Corresponding Quantikine ELISA Results.



Serum samples were obtained from apparently healthy volunteers; no medical histories were available. Serum used to determine GDF-15 correlations were spiked with human HepG2 hepatocellular carcinoma cell culture supernatants to produce sample values across the dynamic range of the standard curve. Samples were simultaneously analyzed using the Luminex High Performance Assay Cardiac Panel A (Catalog # LUCA000) and individually analyzed using the corresponding Quantikine ELISA kits. Quantikine ELISA kits used included the Human CD40 Ligand/TNFSF5 Quantikine ELISA Kit (Catalog # DCDL40), Human GDF-15 Quantikine ELISA Kit (Catalog # DGD150), Human Pappalysin-1/PAPP-A Quantikine ELISA Kit (Catalog # DPPA00), Human Proprotein Convertase 9/PCSK9 Quantikine ELISA Kit (Catalog # DPC900), Human ST2/IL-1 R4 Quantikine ELISA Kit (Catalog # DST200), and Human sTNF RII/TNFRSF1B Quantikine ELISA Kit (Catalog # DRT200). Analyte concentrations determined using each method were graphed. Analyte concentrations determined using the Luminex High Performance Cardiac Panel A or corresponding Quantikine ELISAs are very closely correlated (Goodness of Fit  $R^2 > 0.95$ ).

**Figure 10.** Luminex High Performance Assay Measures Congestive Heart Failure-Related Molecules in Human Serum



The Luminex High Performance Human Cardiac Panel A was used to measure markers of heart failure in human serum samples. Samples collected from individuals with congestive heart failure were purchased (n = 10), and samples from apparently healthy individuals were collected in-house; no medical histories were available (n = 20). PAPP-A was not detectable in any of the samples analyzed.

# Luminex<sup>®</sup> Immunoglobulin Isotyping Assays

Immunoglobulin isotype determination is a valuable step in the study of disease and in the development and characterization of monoclonal antibodies. Luminex Immunoglobulin Isotyping Assays allow for the simultaneous quantitation of up to six immunoglobulin isotypes. Classify Immunoglobulin isotypes in total, or by their kappa or lambda chains in cell culture supernates, serum, or plasma.

## Features

- **Optimized:** All analytes are optimized and tested together for detection of their given analytes in a multiplex setting to ensure the best results possible
- **Flexible:** Measure only what you want! Select one or more of the analytes in a given panel
- **Rapid:** Get more done with results as fast as 1.5 hours
- **Choice:** Polystyrene or magnetic bead formats
- **Options:** Measure total immunoglobulin or investigate the kappa to lambda light chain ratio

## Validation and Testing

Luminex Immunoglobulin Isotyping Assays are validated for use with cell culture supernate, plasma, or serum. They are tested for sensitivity, intra-assay precision, inter-assay precision, and linearity of dilution for all validated sample types. Antibody pairs are tested to confirm that they detect natural samples and the standards provided in the kit equally. This testing is critical for the generation of reliable results as it ensures the accurate determination of target immunoglobulins within biological samples. Assays are also screened to confirm low cross-reactivity with other immunoglobulin isotypes within an assay.

## Kit Contents

- **Base Kits:** Standard cocktails, Kappa light chain detection antibody, Lambda light chain detection antibody, Calibrator Diluent, wash buffer concentrate, streptavidin-PE, filter- or flat-bottomed microplate, mixing bottles, foil plate-sealers, and a standard value card.
- **Beads:** Antibody-coated microparticles.

# Choose Your Luminex® Assay Format

R&D Systems offers Luminex Assays in two formats, the Luminex Assay and the Luminex High Performance Assay. The Luminex Assay is optimized to simultaneously analyze a wide variety and large number of analytes, while the Performance Assays, our most optimized assays, are validated in smaller analyte panels. Use the side-by-side comparison table below to determine which Luminex Assay format is right for you.

Feature		Luminex Assay	High Performance Assay
Species Available	Human	✓	✓
	Mouse	✓	
	Rat	✓	
Sample Volume Required	µL Sample	≤ 50	≤ 100
Multiplex Analyte Capacity	Polystyrene	100 User-Selected	User-Selected from Panel
	Magnetic	50 User-Selected	User-Selected from Panel
Bead Type	Polystyrene	✓	✓
	Magnetic	✓	✓
Validated Sample Types	Serum	✓	✓
	Plasma	✓	✓
	Cell Culture Supernates	✓	See Datasheet*
	Milk		See Datasheet*
	Saliva		See Datasheet*
	Urine		See Datasheet*
Run Time	Hours	3–3.5	2.5–5
Kit Preparation	Premixed	✓	✓
	End-user Mixed		✓
Assay Validation	Mean Sensitivity	< the low standard**	≤ 3/4 the low standard
	Intra-assay Precision	< 20%	< 15%
	Inter-assay Precision	< 25%	< 17%
	Recovery (Individual Samples)	N/A	70–130%
	Assay Linearity	Dose-Dependent Decrease in Values	1:2–1:8
	Linearity (Individual Samples)	Dose-Dependent Decrease in Values	70–130%
Analytes Available	Human	>340	>100
	Mouse	92	0
	Rat	17	0

\*Visit [rndsystems.com/LuminexHighPerformance](http://rndsystems.com/LuminexHighPerformance)

\*\*Visit [rndsystems.com/LuminexAssay](http://rndsystems.com/LuminexAssay) for mean sensitivity values for each analyte

## Ordering Your Luminex® Assay

The R&D Systems Luminex Assay Online Ordering Tool is designed to help you choose the right Luminex Assay for your unique research needs.

The ordering tool directs you step-by-step through the selection of:

1. Assay type (Luminex High Performance or Luminex Assay)
2. Bead type (polystyrene or magnetic)
3. Species options (human, mouse, rat)
4. Separate by sample dilution factor\*
5. Available analytes\*

\*Information concerning analytes with potential assay cross-reactivity or overlapping bead regions are noted within the ordering tool to facilitate the selection of optimized user-defined multiplex assays.

### Additional Features

The R&D Systems Luminex Assay Online Ordering Tool includes these additional features to facilitate ordering:

- Saving and retrieving your selected Luminex Assay configuration makes re-ordering quick and easy
- View how the selection of each assay specification changes the final kit options
- Find pricing options for premixed or end-user mixed High Performance Assays
- Easy navigation within the tool allows you to alter your assay selections at any time
- Access to ordering or technical support from representatives via phone and email

Access the Luminex Assay Online Ordering Tool at  
[rndsystems.com/Luminex](https://rndsystems.com/Luminex)

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