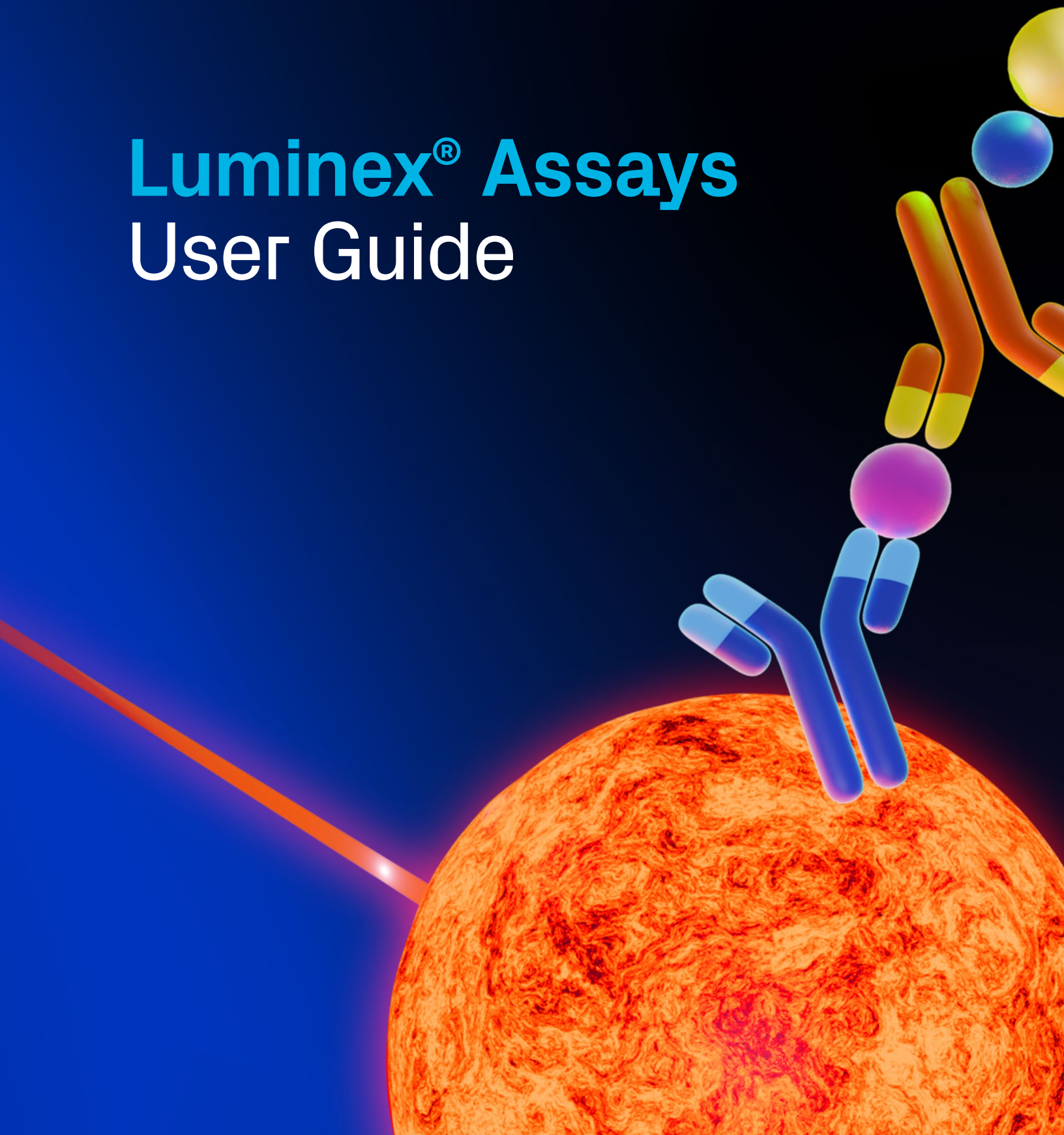
bio-techne® | R&D SYSTEMS

Luminex® Assays
User Guide



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What are the Benefits of Multiplexing?

To put it simply, multiplexing allows multiple biological target analytes to be simultaneously examined and quantified in a single sample. The simultaneous analysis of multiple factors provides numerous research advantages.

- **Maximizes Limited Sample:** data collection from just 25 µL or less of undiluted sample.
- **Minimizes Experimental Variability:** samples are processed only once, so multiple data points are derived from a single manipulation.
- **Optimizes Productivity:** minimal sample preparation and processing; whilst generating high volumes of data.
- **Economical:** examining multiple analytes in a single sample saves time and resources.

What is a Luminex Multiplex Immunoassay?

A Luminex assay is a magnetic microparticle-based immunoassay which utilizes the same sandwich principles as traditional ELISAs. Luminex multiplex immunoassays allow you to quantify up to 100 biomarkers with less sample volume than a traditional plate-based ELISA. Color-coded microspheres, or beads, are internally dyed with different proportions of red and infrared fluorophores that correspond to a distinct spectral signature, or bead region.

Antibodies specific to a desired analyte are coupled to a unique bead region and are incubated with a sample. After washing away unbound materials, beads are incubated with a mixture of biotinylated detection antibodies and a streptavidin-phycoerythrin (PE) reporter. Using a Luminex instrument, beads are excited by one laser or LED, depending on the instrument, to determine the bead region and corresponding assigned analyte. Another laser or LED determines the magnitude of the PE-derived signal, which is proportional to the amount of analyte bound. Multiple readings are taken at each bead region, ensuring robust detection.

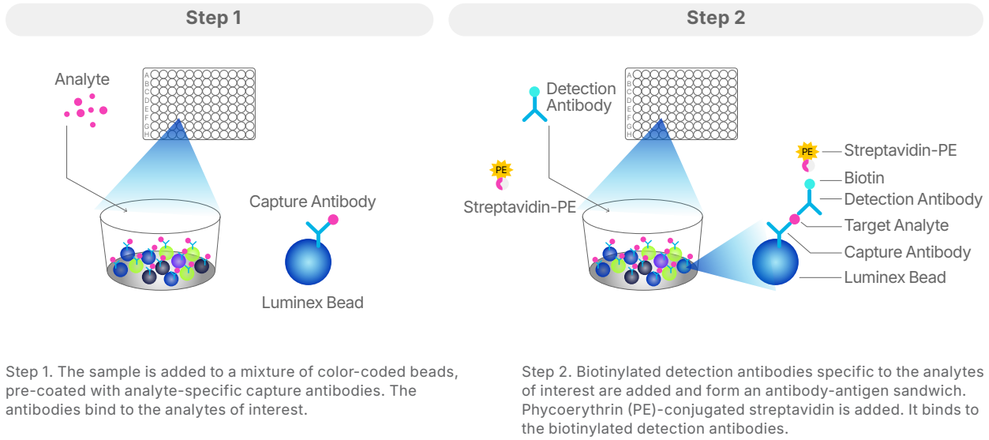
Why Use R&D Systems Luminex Multiplex Immunoassays?

Your results matter, so what's inside your Luminex multiplex immunoassay should too! Our **Luminex assays** utilize R&D Systems™ antibodies and proteins, same as our ELISA kits and are rigorously tested for suitability for multiplex immunoassay applications.

- **Save Time and Sample:** Quantify up to 50 analytes with 25-50 µL of sample.
- **Large Flexibility:** Customize your Luminex panel with the analytes you want to measure.
- **Unparalleled Accuracy, Precision and Sensitivity:** High Performance Panels are correlate with the gold standard Quantikine® ELISAs.
- **Consistency:** All assays undergo rigorous quality control, so results correlate day-to-day, experiment-to-experiment.
- **Extensive Analyte Portfolio:** Experiment with >490 unique targets spanning human, mouse, rat, porcine, and non-human primate.

FIGURE // 01

Luminex Assay Principle



Step 1. 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.

Step 2. Biotinylated detection antibodies specific to the analytes of interest are added and form an antibody-antigen sandwich. Phycoerythrin (PE)-conjugated streptavidin is added. It binds to the biotinylated detection antibodies.

Step 3. A magnet in the Luminex instrument 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. Each well is imaged with a CCD camera.

Which Luminex Multiplex Immunoassay is Right for Me?

R&D Systems offers two bead-based multiplex immunoassay options utilizing Luminex xMAP® microparticle technology. Our immunoassays help you tailor assay selection to your individual research needs. Please note that the sample dilution and the number of standards used can influence plex size and configuration. Design the multiplexing assay you need for your preliminary or discovery investigations with the R&D Systems Luminex Discovery assays. These assays are optimized to simultaneously analyze a wide variety and large number of analytes. Next, take your research to the next level by customizing one of our Luminex High Performance panels to get accurate and precise quantitation which deliver near single-analyte ELISA performance.

TABLE // 01

R&D Systems Luminex Assay Offerings

	Luminex Discovery Assays	Luminex High Performance Assays
What	Biomarker discovery or screening	Biomarker verification
When	Early stage	Later stage
Why	Quantitative	Quantitative
	Economical	High sensitivity
	Maximum multiplexing capacity	Greatest accuracy and performance. Validated and correlated against R&D Systems Quantikine ELISA
	Most flexible option with mix and match selection	Pre-defined fixed and configurable with in panels

TABLE // 02

R&D Systems Luminex Discovery & High Performance Assays

	Luminex Discovery Assays	Luminex "Premixed" High Performance Assays	Luminex "User Mixed" High Performance Assays	Luminex Fixed High Performance Assays
Maximum Analyte Multiplex	50	46	46	46
Sample Volume (µL)	25-50	25-50	25-50	25-50
Microparticles Premixed	Yes	Yes	No	Yes
Biotin Antibodies Premixed	Yes	Yes	No	Yes
Controls Available	No	Yes	Yes	Yes
Premix QC Prior to Shipping	Yes, full QC	Yes, Confirmational QC	Yes, Confirmational QC	Yes, Confirmational QC
# Analytes Available	>450	>100	>100	100
Species Available	Human, Mouse, Rat, Porcine	Human, Mouse, Primate	Human, Mouse, Primate	Human, Mouse
Validated Sample Types	Cell culture supernatants, serum, EDTA plasma, Heparin plasma. For mouse analytes only, tissue lysates.	Cell culture supernatants, serum, EDTA plasma, Heparin plasma; For select panels**: saliva, urine, milk.	Cell culture supernatants, serum, EDTA plasma, Heparin plasma; for select panels**: saliva, urine, milk.	Same as “Premixed”
Analyte Selection	User defined	Predetermined but customizable.	Predetermined but customizable.	Fixed.
Assay Format	All required components included.	All required components included.	Bead sets and base kits separate.	All required components included.
Delivery Time	U.S. orders ship in 7 business days.	U.S. orders ship in 5 business days.	U.S. orders ship in 2 days.	U.S. orders usually ship in 1 day.
# Panels Available	Custom	10 customizable panels, including 2 high sensitivity panels.	13 customizable panels, including 2 high sensitivity panels.	9 fixed panels.

Start Building Your Panel

Scan the QR Code or Contact:
rndsystems.com/luminex/analytes

Ensuring Luminex Performance & Consistency

R&D Systems Luminex assays outperform competitor assays in many dimensions, including natural sample linearity, lot-to-lot consistency, precision and reproducibility, sensitivity, and minimization of false positive signals.

Accurate Detection of Natural Proteins

Antibody pairs recognize both the supplied recombinant standard and the natural proteins in biological samples. Natural sample linearity (sometimes referred to as parallelism) is a hallmark of the R&D Systems Luminex High Performance assay and confirms that the kit can accurately measure the relative mass values of the natural analyte. R&D Systems also determines the ideal standard curve range for each assay, ensuring optimal sensitivity and reproducibility of results.

FIGURE // 02

CCL2/MCP-1 beta Spike Linearity

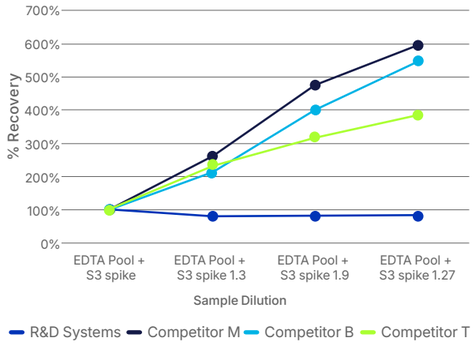


Figure 2. MCP-1 (**Catalog # LUXLM279**) natural sample linearity is maintained with the R&D Systems® recombinant standard but is not maintained with the competitor assays.

Confirmed Lot-to-Lot Consistency

Another hallmark of the R&D Systems Luminex High Performance products is that all lots are tested to ensure low background, consistent standard curves and dynamic ranges. Each standard is anchored to the same master calibrator to ensure that sample data is comparable over time.

FIGURE // 03

IL-6 Controls

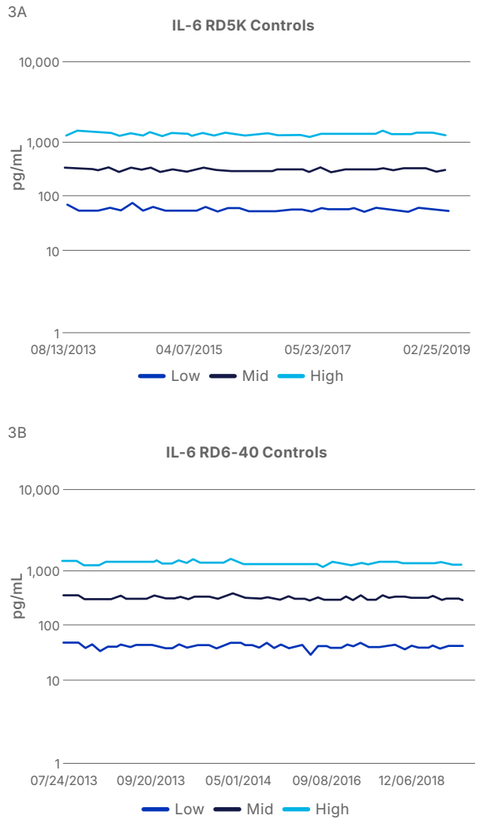


Figure 3. Levey-Jennings control plots show IL-6 (**Catalog # LUXLM206**) lot-to-lot consistency in RD5K diluent (3A) and RD6-40 diluent (3B) over the course of 6 years.

Precision & Reproducibility

Providing Confidence in Your Results

Immunoassay precision is defined as the reproducibility of results within and between assays. This characteristic of an immunoassay is extremely important in order to: 1) provide assurance that the results obtained throughout a study are accurate and reproducible from one experiment to the next and 2) determine if two results are the same or different. Precision is measured as a coefficient of variation (CV) from the mean value. Two types of precision should be considered, intra-assay precision and inter-assay precision. Intra-assay precision is the reproducibility between wells within an assay.

This allows you to run multiple replicates of the same sample on one plate and obtain similar results. Inter-assay precision refers to the reproducibility between assays, ensuring consistent results when using multiple kits over time. R&D Systems Luminex Assays typically have CV values less than 30% across the standard curve for both intra- and inter-assay precision, while the High Performance Assays typically have CV values less than 17%. These low CV values allow you to perform repeated assays and be confident that the results are consistent throughout the study.

TABLE // 03

R&D Systems Luminex Assays CV Values

Analyte	Intra-Assay (%CV)	Inter-Assay (%CV)
BDNF	8.16	13.3
CCL2/MCP-1	3.02	10.5
CCL5/RANTES	3.72	17.0
CCL11/Eotaxin	7.98	15.4
CCL20/MIP-3a	8.41	17.3
CD40 Ligand	9.30	15.0
CXCL2/GROϕ3	7.76	13.1
CXCL10/IP-10	2.95	12.2
CXCL11/I-TAC	6.23	13.7
CXCL13/BLC	5.79	12.5
FGF basic	5.60	13.1
G-CSF	5.55	14.2
GM-CSF	7.11	14.1
Granzyme B	9.75	18.6
INF-α	5.17	12.4
INF-ϕ3	10.9	15.2
INF-γ	6.36	13.0
IL-1ϕ3	2.55	12.7

Analyte	Intra-Assay (%CV)	Inter-Assay (%CV)
IL-10	8.55	14.1
IL-12 p70	4.92	17.1
IL-13	7.97	17.5
IL-15	4.99	18.2
IL-17A	4.38	19.0
IL-2	5.32	18.1
IL-21	5.84	19.0
IL-4	4.92	17.5
IL-5	4.14	16.5
IL-6	6.80	17.8
IL-7	6.57	17.7
IL-8/CXCL8	6.87	17.5
PDGF-AA	6.52	25.0
PDGF-BB	3.37	16.9
PD-L1	8.36	19.4
TGF-α	5.43	18.7
TNF-α	3.68	17.2
VEGF	4.79	18.4

Table 3. Data from the Non-Human Primate XL Cytokine panel (**Catalog # FCSTM21**) indicate that all analytes have an intra assay CV below 11% from 40 reportable results and an Inter-assay CV below 26% across 31 assays.

Sensitivity

The minimum detectable dose is the lowest measurable value that is statistically different from zero. It is calculated by adding two standard deviations to the mean optical density value of several zero standard replicates and determining the corresponding analyte concentration from the standard curve.

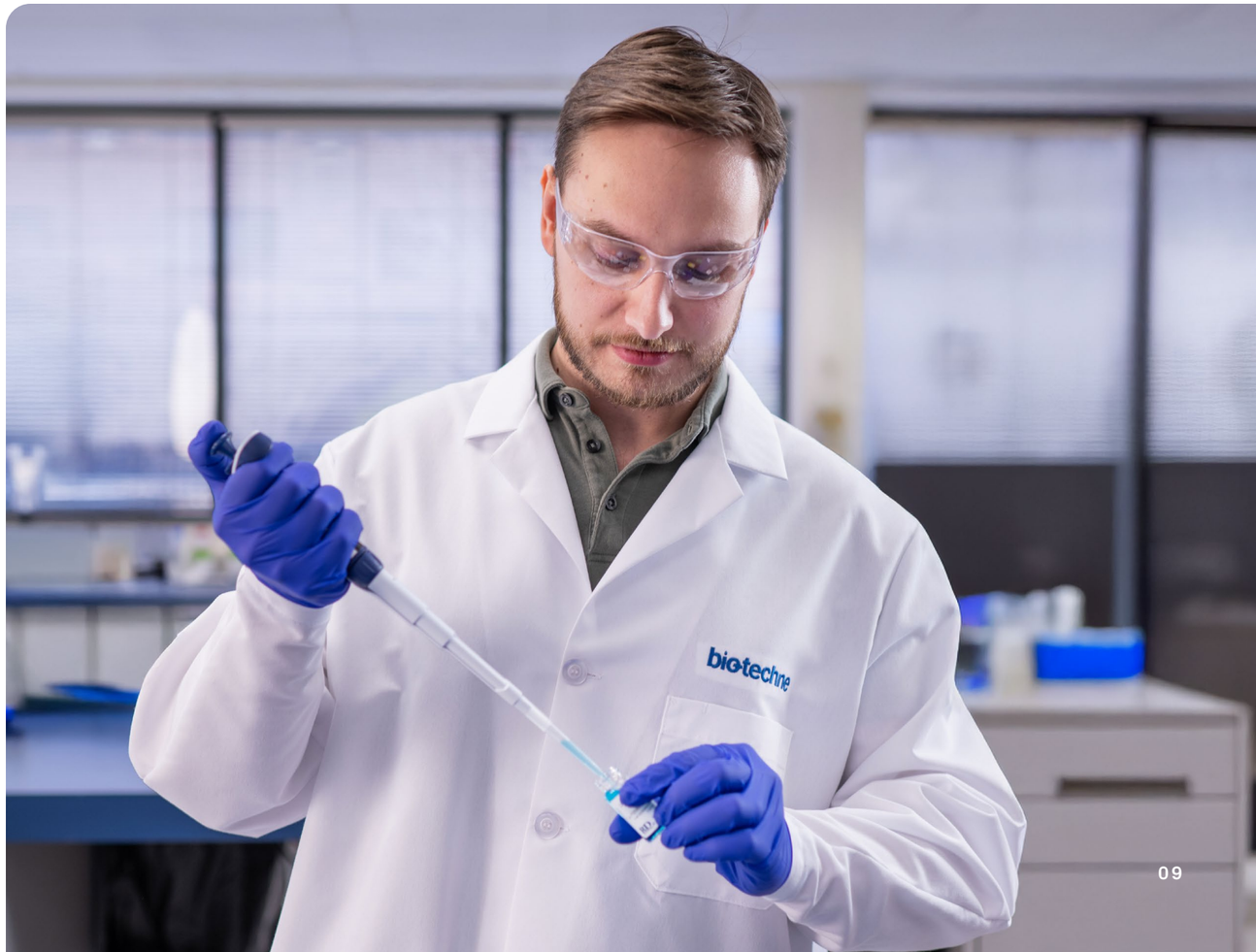
The better the sensitivity of an assay, the lower the useful working range (standard curve range) will be. R&D Systems Luminex Assays and High Performance Assays are optimized to ensure high signal, low background, and the best sensitivity possible.

TABLE // 04

R&D Systems Luminex Assays Sensitivity

Analyte	Mean (pg/mL)	Range (pg/mL)
CA15-3	12.3	9.83-14.5
Chromogranin A	16.6	12.1-19.7
HE4	2.85	1.86-4.32
MIF	59.8	34.2-79.1

Table 4. Examples of minimal detectable dose (MDD) from the Human Tumor Biomarker Luminex Performance assay (**Catalog # FCSTM25**). Nine assays were run and the MDD was determined by adding 2 standard deviations to the MFI of twenty zero standard replicates and calculating the corresponding concentration.



Linearity Experiments Identify False Positive Signals

A false positive result incorrectly detects an analyte not detectable in a given sample. Linearity of dilution is commonly used to identify false positives. Sample values should remain consistent when running multiple dilutions and back-calculating concentration. If sample values increase with increasing dilutions, this indicates a specificity issue. R&D Systems Luminex Assays and High Performance Assays are built, split if necessary, to prevent false positives.

FIGURE // 04

R&D Systems Luminex Assays Sensitivity

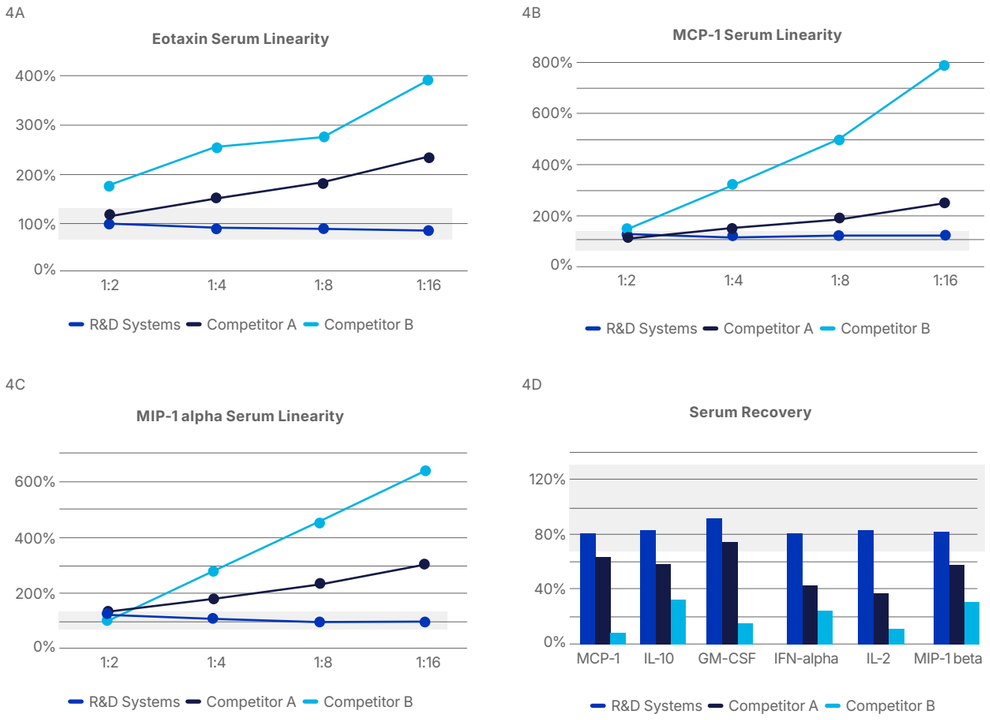


Figure 4. Serum linearity for Eotaxin, MCP-1, and MIP-1 alpha are shown in figures 4A, 4B, and 4C respectively. A known amount of protein was added to serum samples and serially diluted. R&D Systems Human XL panel was tested side by side with two of the leading competitors. The gray zones indicate the acceptable range of expected values within the range of 70% - 130%. Serum recovery rates are shown in figure 4D. A known amount of protein was added to serum samples and the recovery rates were measured. R&D Systems Human XL panel was tested side by side against two of the leading competitors. Again, gray zones are included to indicate the acceptable range of expected values of ±30%.

Sample Preparations

The sample collection and storage conditions listed below are intended as general guidelines and is recommended you refer to the Product Datasheet of your assay for specific guidelines. Samples require a minimum 2-fold dilution. High abundance biomarkers may require additional dilution for samples to meet performance criteria such as being within the dynamic range of the assay. Sample stability has not been evaluated. Hemolyzed, icteric and lipemic samples are generally not suitable for Luminex assays. Note that each of the following sample types have not been validated for all Luminex assays. Check the kit insert to determine validated sample types.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Lysates – Rinse tissue with PBS and cut into 1-2 mm pieces. Homogenize with a tissue homogenizer in PBS. Add an equal volume of Cell Lysis Buffer 2 (**R&D Systems, Catalog # 895347**) and lyse at room temperature for 30 minutes with gentle agitation. Remove debris by centrifugation. Assay immediately, or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Platelet-poor Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge at 16,000 x g for 4 minutes to remove particulate matter. Assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Standard Assay Procedure

1. Prepare all reagents as instructed.
2. Add 50 µL of standard, control, or sample* to each well. *Samples require dilution.
3. Add 50 µL of diluted microparticle cocktail to each well. Incubate for 2 hours at RT on a shaker at 800 rpm.
4. Wash by removing the liquid from each well, filling with 100 µL Wash Buffer, removing the liquid again. Perform the wash 3 times.
5. Add 50 µL of diluted Biotin-Antibody Cocktail to each well. Cover and incubate for 1 hour at RT on the shaker at 800 rpm.
6. Repeat the wash as in step 4.
7. Add 50 µL of diluted Streptavidin-PE to each well. Incubate for 30 minutes at RT on the shaker at 800 rpm.
8. Repeat the wash as in step 4.
9. Add 100 µL of Wash Buffer to each well. Incubate for 2 minutes at RT on the shaker at 800 rpm.
10. Read within 90 minutes using a Luminex Instrument **Note:** Resuspend microparticles immediately prior to reading.

Data Analysis: Calculation of Results

Calculating Concentration of Target Protein in the Sample

The values of the unknown samples are assigned in relation to the standard curve. Since samples are diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Always run samples in duplicate or triplicate, to provide enough data for statistical validation of the results. Average the duplicate or triplicate readings for each standard, control, and sample and subtract the average zero standard (blank) median fluorescence intensity (MFI). The coefficient of variation (CV) of duplicates should be ≤ 20%.

Create a standard curve by using dedicated software or R&D Systems **Quantist Analysis Software** capable of plotting the mean MFI (y axis) against the protein concentration (x axis) and generating a five-parameter logistic (5-PL) curve-fit.

Calculating the Coefficient of Variation

The coefficient of variation (CV) is the ratio of the standard deviation to the mean, which is usually expressed as a percentage.

Best Practices

- Make sure all reagents are brought to room temperature before using (unless instructed to keep them cold).
- Standards and controls are single-use and should not be aliquoted or frozen after reconstitution.
- Multichannel pipettes speed the ability to plate your standard and samples and lead to more consistent results. When pipetting, dispense liquid with the pipette tips held at an angle and avoid touching the bottom of the well.
- While it is not necessary to change your pipette tips between each replicate, it is recommended that you change them between different samples or standards to prevent contamination.
- It is highly recommended that automatic plate washer is used to achieve the most consistent assay results.
- When washing plates, either manually or with a plate washer, be sure to give the wash buffer time to work by adding a 30 second soak time in between washes.
- Pay close attention to the incubation times. As a general guide the incubation time should not vary by more than +/- 5 minutes per hour of incubation time.
- On the day of the assay, all fresh and previously frozen serum and plasma samples require centrifugation at 16,000 x g for 4 minutes immediately prior to use or dilution.

Common FAQS

What is included in an R&D Systems Luminex Assay Kit?

Pre-mixed Luminex Assays are a complete kit consisting of:

- Precoated microparticle cocktails
- Biotinylated detection antibody cocktails
- Standards
- Diluents
- Calibrator diluent
- Assay diluent
- Streptavidin-PE concentrate
- Wash buffer
- Plate and plate sealers.
- QR code for assay instructions

User mixed Luminex High Performance Assays are supplied as a base kit containing all of the reagents necessary to run the assay with separate microparticle and biotinylated detection antibody concentrates.
Note: Some users mixed High Performance Assay kits, such as the XL panels, will have a lyophilized biotinylated antibody cocktail supplied with the base kit and will not contain a “matched set” of microparticles and detection antibody.

How many samples can be run in a Luminex Assay Kit?

Typically, 40 samples can be assayed in duplicate. This will depend on the number of points being evaluated for the standard curve and the inclusion of any controls. The R&D Systems Luminex Assay is typically run with a six-point standard curve and High Performance Assays are run with a seven-point standard curve. Please refer to the datasheet for details that may be specific to your kit.

What samples can be tested in the kit?

Most R&D Systems Luminex kits are validated for sera, plasma (EDTA or Heparin), and cell culture supernate. However, the samples validated can vary from product to product. The product datasheet and product-specific web page states all sample types that have been validated for use with the specific kit. These are the only samples for which we can support the claims and support. Unvalidated sample types should be validated independently with a spike and recovery study (details below).

Has this kit ever been tested with my sample type?

R&D Systems has not routinely tested all sample types such as tissue homogenates for Luminex kits. This does not mean that the Luminex kit is not suitable for other sample types. Each investigator will need to perform a spike and recovery study to determine if an unvalidated sample type will work with the kit. To perform a spike and recovery experiment, divide a sample into two aliquots. In one of the aliquots, spike in a known amount of the kit standard. A dilution series is performed comparing the spiked versus the unspiked sample. This method may be used to validate any sample type that has not been evaluated by
R&D Systems. For a more detailed spike and recovery protocol, please contact **Technical Service**.
Note: Acceptable ranges should be determined individually by each laboratory.

Why can't I detect any of my samples?

You will be able to quantify samples down to the lowest point on the standard curve. In some cases, the standard curve does go down low enough to detect normal samples. You can check the sample values section of the analyte specific datasheet to find what kind of sample values we obtained from apparently healthy individual donors. You may also want to review the literature to find out if there is an established normal range for your target. It is important to recognize that assay platforms and manufacturers differ in their calibrations for their unique assay products and reported measurements may not directly correlate.

Can I extend the standard curve (in either direction)?

R&D Systems cannot support kit results outside the stated range under any circumstances. A specific range was chosen because of confidence in the reproducibility of the assay.

What is assay sensitivity?

Sensitivity is the lowest measurable value that is statistically not equal to zero. It is calculated based on the signal of the background and the inherent variability of the assay. It is commonly determined by taking the mean fluorescence intensity (MFI) plus two standard deviations from 20 zero replicates. This value is converted into analyte concentration from the standard curve. The low standard is the lowest point that the value is in the linear portion of the standard curve and, therefore, quantifiable. Values which are greater than the sensitivity can be distinguished as separate from the background or the noise of the assay, however the confidence level for reporting these values is lower than if the sample values fall within the standard curve range.

Why is a sample dilution necessary?

In some assays most samples read above the standard curve, thus requiring a dilution for analyte levels to fall within the range of the assay. Another reason for dilution is to limit interference due to factors in complex matrices.

Will addition of an assay diluent cause further dilution of the sample?

Since the assay diluent is added to all wells, standards and specimens are treated equally. Therefore, sample concentration can be read from the standard curve without adjusting for this dilution.

Is there enough calibrator diluent for all my sample preparations?

The kits are designed with enough calibrator diluent to ensure that the vast majority of samples fall within the indicated range of the assay. Should you find that there is not enough diluent provided in the kit to dilute your samples, you have at least two options. Option 1: Samples can be diluted in two steps. The initial dilution in culture medium and a final dilution, of at least 1:10, into the Calibrator Diluent provided in the kit. Option 2: You can purchase additional diluent provided the same lot included in the kit is still available. Contact Technical Service for more information.

My diluents appear to contain precipitate, is this okay?

Due to saturating amounts of some buffer components, some of the RD1 Assay Diluents contain a light to heavy precipitate. This should not affect the assay. In these instances, it will be noted in the specific protocol booklet. If it is not noted in the protocol booklet, please contact Technical Service.

The assay protocol specifies the use of a shaker at 800 rpm. This is too fast for my shaker. Is this correct?

This is 800 rpm with a 0.12 orbit. If the plate shaker has a larger orbit, a lower speed should be used.

Are controls available for kits?

R&D Systems Luminex Discovery Assays do not have kit controls available for purchase. However, R&D Systems Luminex High Performance Assays either include kit controls or are available as supplemental products. Please inquire for specific ordering information.

What is the stability of supplemental controls?

Controls are assigned an expiration date of 6 months from date of receipt. They are to be used once and discarded. If the lyophilized controls are stored properly, it is possible that they will remain stable for an extended period of time, although we have not conducted extended stability testing. The controls have not been tested for stability after reconstitution.

I used your recombinant protein as a control in the corresponding R&D Systems Luminex Assay Kit. Why am I seeing discrepancy in mass values?

A large dilution is required to place the recombinant protein on the standard curve range. Typically, this is a dilution from µg/mL to pg/mL. Any dilution step can introduce inaccuracy and the larger the dilution step the greater the potential for error. Any pipetting error or mis-calibrated pipet can result in apparent over- or under-recovery. R&D Systems Luminex Assays have been developed to measure a level of protein captured by one antibody and detected by a second antibody. This measurement is calibrated to standards established when the kit was initially developed. The protein determination of these initial standards became the Master Calibrators to which all new standards are formulated. This provides R&D Systems Luminex Assay kits with consistency between manufacturing lots. We would expect +/- 30% recovery of the amount stated on the vial when using the Luminex Assay to determine a protein concentration. There may be slight differences in the immunologically recognizable mass between lots of protein, so the apparent concentration provided on the vial may vary from lot-to-lot when measured in the immunoassay. If you are using proteins to make controls, it is better to value assign the mass based on measurement from the kit and not use the mass on the vial when setting control levels.

Why must I use polypropylene tube for standard curve dilutions?

Certain proteins or analytes will bind to glass and polystyrene, but do not readily bind to the polypropylene tubes.

Can I run a partial plate?

While it is possible to run less than a full plate and retain the microparticle or biotinylated detection antibody cocktails, this only applies to the concentrates. A fresh working stock of material (microparticles, biotinylated detection antibody, standards and controls) should be generated at the time of assay. Lyophilized standards and controls are single use only and should be freshly prepared at the time of assay.

Can I adjust the incubation times or temperatures from the instructions in the kit insert?

R&D Systems has optimized the assays for both incubation times and temperatures. Each kit has been validated for the protocol described in the kit datasheet. We cannot guarantee the performance of our kits when the protocol has been altered in any way.

Can reagents from different kits be interchanged?

Assay Diluent(s), Calibrator Diluent(s), and other kit components may only be interchanged if they have the same part number AND lot number. R&D Systems does “whole kit QC” which means that we cannot support the use of reagents from other lots or sources being substituted into an assay.

Why am I seeing high variability between sample duplicates?

High intra-assay variability can be caused by poor pipetting and/or poor washing technique.

TABLE // 05

Troubleshooting Guide

Observation	Possible Source	Suggestions
Acquisition Problems & Error Messages	Incompatible instrument	Use the instrument that is compatible to the microparticle type. R&D Systems Luminex assays are compatible with all Luminex instruments.
	Instrument is out of calibration	To obtain accurate measurements, regular calibration of the instrument is required. Best practice is to run assays within one week of calibration. Luminex Corporation recommends running verification the day of the assay to confirm the instrument is functioning properly and is within current calibration settings. Perform instrument calibration and verification per the instrument user’s manual.
	Incorrect probe height	Adjust the sample probe vertical height and align to the plate per the instrument user’s manual.
	Sample probe is clogged	Clean the sample probe per the instrument user’s manual. Replace the sample probe if necessary. Remember to readjust the vertical height each time the probe has been removed.
	Microparticle spectral address is not assigned correctly	Ensure microparticle regions are assigned correctly per the kit insert or the Certificate of Analysis (CofA). Microparticle maps are custom created depending upon the analytes selected. In the event of omission or an incorrect assignment of a microparticle region, data will be missing in the CSV file, try the “Replay” function to retrieve the data following selection of the appropriate microparticle regions.
	Incorrect instrument settings	Follow the insert instructions on instrument settings.
Low Microparticle Count	Instrument is out of calibration	Perform instrument calibration and verification. To obtain accurate measurements regular calibration of the instrument is required. Best practice is to run assays within one week of calibration. Luminex Corporation recommend running verification the day of the assay to confirm the instrument is functioning properly with current calibration settings.
	Wrong event or microparticle setting	Verify that the events/bead is set at 50. A 50-count per analyte is sufficient to produce a statistically accurate result. A bead count of about 25 may be acceptable if the samples are run in duplicates and other parameters of measuring the performance of the assay are fine.
	The system is timed-out (Luminex 100/200™ and FLEXMAP3D™)	If your instrument times out when using flow cytometry-based instruments such as the Luminex 200, stop the plate run. Check the probe height and confirm the magnetic microparticle type is selected. Then re-run the plate. As each microparticle has a different rate for acquisition and the instrument is set to collect 50 microparticles in a designated time, a “time-out” may result in insufficient microparticle counts for one or more analytes.
	Sample contains debris which affects acquisition	Centrifuge samples on the day of the assay at approximately 16,000 x g for 4 minutes immediately before use. In rare cases, an extended centrifugation may be necessary.
	Miscalculation of microparticle dilution/ lower number of microparticles added per well	Confirm microparticles were diluted according to the kit insert.

TABLE // 05

Troubleshooting Guide Continued

Observation	Possible Source	Suggestions
Low Microparticle Count	Microparticles are clumped or aggregated	Centrifuge the microparticle cocktail concentrate (for 30 seconds at 1,000xg) and gently vortex the concentrated before preparing the 1X diluted microparticle cocktail.
	Microparticles not in suspension during acquisition	Immediately before placing the plate on the reader, shake the plate for one additional minute in 1X Wash Buffer to resuspend the microparticles.
	Shaker with incorrect settings	Use a horizontal orbital microplate shaker with a 0.12" orbit. Ensure the shaker speed is set per recommendations from the kit insert.
	Magnetic microparticles not collected at the bottom of plate during wash steps	Use an appropriate magnetic device designed to accommodate a microplate. Wash by applying the magnet to the bottom of the microplate, allow 1 minute before decanting wash buffer. Do not blot dry as this may cause a loss of microparticles.
	Sample was run undiluted	Samples require at least a 2-fold dilution with the appropriate Calibrator Diluent. Mix thoroughly. Samples may require higher than 2-fold dilutions. Review the Product Insert, Certificate of Analysis or R&D Systems® Luminex Assay Customization Tool for the suggested starting dilution for each sample type.
	Blockage of sample probe	See above under acquisition and error messages.
Low Fluorescence Intensity (FI) Signal or Poor Sensitivity	Non-optimal preparation of the standard curve	Confirm the standard reconstitution volume from the standard value card or the Certificate of Analysis. Incorrect reconstitution of the standard will result in inaccurate sample value calculations. Be sure to follow reconstitution instructions for all lyophilized reagents outlined in the kit insert.
	Non-optimal dilution of the detection antibodies or streptavidin-PE concentrates	Confirm reagent dilutions were performed according to the kit insert.
	Photo-bleaching of the PE signal	Streptavidin-PE is light sensitive. Protect from light.
	Incorrect shaker settings	See above.
	Incorrect instrument settings	See above.
Sample Readings are Out of Range (OOR Error Message)	Samples are below assay range (<OOR Error Messages) and contain no analyte, or the analyte level is below the level of detection, or the sample may be too diluted.	Analyte of interest may be undetectable in the assay range due to low abundance of natural protein. Check kit instructions or the R&D Systems Luminex Assay Customization Tool for suggested sample dilution. Suggested dilution factors are based on samples from healthy volunteers. Depending on the unique nature of an individual sample, a different dilution factor may be needed to bring the reading within the dynamic range of the assay.
	When readings are above assay range (>OOR Error Messages)	Review the Product Insert, CofA or Check kit instructions or the R&D Systems Luminex Assay Customization Tool for the suggested initial dilution. Suggested dilution factors are based on samples from healthy volunteers. Depending on the nature of the sample, it may require a further dilution to bring the reading within the assay range.
		Note - Samples may require dilution and re-analysis if a specific analyte is out of range.

TABLE // 05

Troubleshooting Guide Continued

Observation	Possible Source	Suggestions
Poor Precision with Sample Measurements	Presence of interfering components in samples, especially samples with complex matrices such as plasma and serum	Check for the presence of interfering components, additives, or if gel separators were introduced into the sample by performing a Spike/Recovery and Linearity test. Contact R&D Systems Technical Service if you require assistance with this test.
	Sample type not validated for the assay	Check the kit insert to confirm if the sample type has been validated for the assay.
	Samples with hemolyzed and hyperlipidemic matrices	Avoid the use of samples with hemolyzed or hyperlipidemic matrices. Such samples may disrupt antibody binding or clog the probe. See discussion above on how to clean the sample probe.
	Integrity of the sample is compromised while in storage	Follow the kit insert on Sample Collection & Storage. Observe best practices for processing and storing the samples after collection. Avoid repeated freeze-thaw cycles.
	Non-optimal pipetting technique	Ensure a consistent and accurate pipetting method. Dispense microparticles, diluents and samples accurately. Change pipette tips between samples and dilutions. Pre-wet tips for sample replicates. Ensure that your pipettes are calibrated regularly.
	Assay reagents not equilibrated to room temperature prior to use	All assay components should be equilibrated to room temperature prior to use.
High Background Signal	Incorrect buffer used for the dilution of standards and/or samples	Ensure the use of the recommended Calibrator Diluent for the dilution of standards/samples per kit insert.
	Blank wells accidentally spiked with standard or samples	Do not add standard or samples to wells designated as blank. Add Calibrator Diluent only.
	Extended incubation with detection Abs or streptavidin-PE	Follow the kit instructions for incubation times and follow precisely.
Microparticle Aggregation	Samples with hemolyzed and hyperlipidemic matrices	See above
	Microparticles not thoroughly mixed	Follow the kit instructions on the preparation of the diluted microparticle cocktail. Use a plate shaker with appropriate settings for the assay. Shake plate for one additional minute in 1X Wash Buffer immediately before analyzing in an appropriate instrument.
	Doublet Discriminator gates setting is incorrect	Check the kit insert for the Doublet Discriminator gate settings and adjust settings as needed.

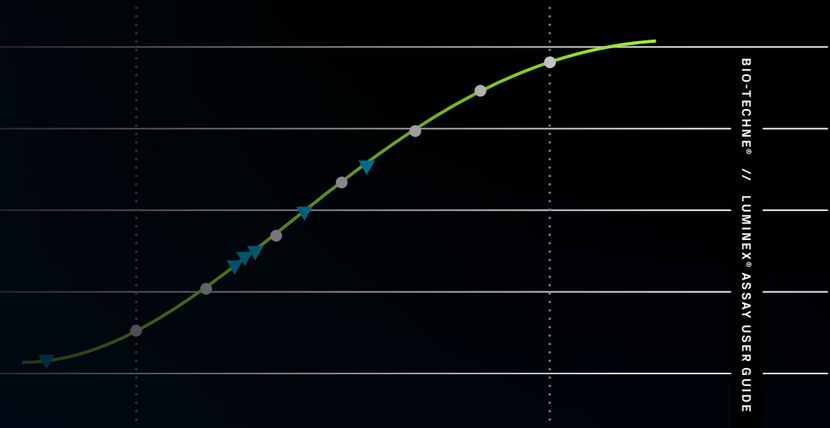
Luminex Instrumentation

Luminex instruments offer proven precision and reliability, making them among the most widely used multiplexing platforms in the market. Upon completion of an R&D Systems Luminex assay, beads are analyzed with a dedicated dual-laser flow-based detection instrument. From the Luminex 200 to the advanced xMAP INTELLIFLEX, choose the Luminex instrument that best meets your unique application requirements.

TABLE // 06

Luminex Instruments Comparison

	Luminex 200 Instrument System	Luminex FLEXMAP 3D Instrument System	Luminex xMAP INTELLIFLEX System	Luminex xMAP INTELLIFLEX DR-SE System
Number of Targets Multiplex Capacity	Up to 100	Up to 500	Up to 500	Up to 500
Read time for 96 Well Plate	~40 minutes	~20 minutes	~20 minutes	~20 minutes
Read time for 384 Well Plate	N/A	~75 minutes	~75 minutes	~75 minutes
Dynamic Range	3.5 logarithmic units	4.5 logarithmic units	≥5.5 logarithmic units	≥5.5 logarithmic units
Microtiter Wells	96	96 and 384	96 and 384	96 and 384
Dimensions (W x D x H)	25.3″ x 23.5″ x 12.5″ 64cm x 60cm x 32.5cm	23″ x 25.7″ x 18″ 58.4cm x 63.5cm x 45.7cm	23″ x 24″ x 30″ 58.4cm x 61cm x 76.2cm	23″ x 24″ x 30″ 58.4cm x 61cm x 76.2cm
Touchscreen	N/A	N/A	Yes	Yes
Automated Startup	N/A	N/A	Yes	Yes
Reporter Laser	532 nm (green)	532 nm (green)	532 nm (green)	532 nm (green) and 405 nm (violet)
Dual Reporter Readout	N/A	N/A	N/A	Yes
Barcode Reader for Calibration and Verification Reagents	N/A	N/A	Yes	Yes



Analysis Software

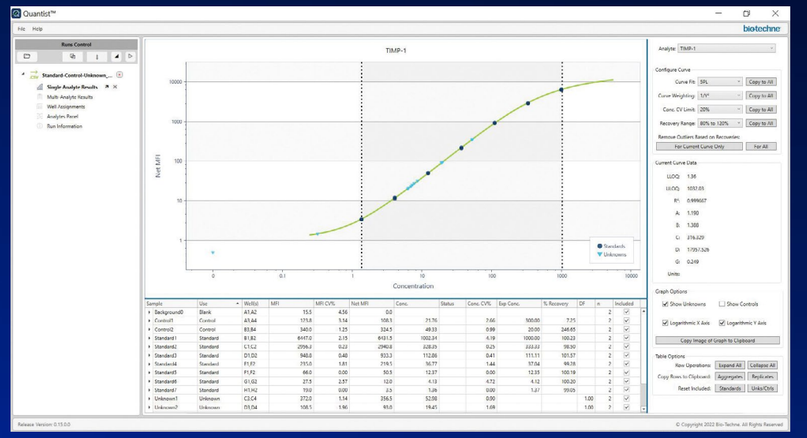
Quantist™ Luminex data analysis software helps you extract valuable insights from data complexity. Use this software to evaluate the quality of your standards, controls, and unknowns. Evaluate the long-term consistency of your Luminex data by using Quantist to make inter-assay comparisons. Import CSV files from any Luminex instrument and export ready-to-graph, Excel files.

- Easily exclude individual wells or aggregates
- Remove low bead counts with a single click
- Assign or update well assignments in the Luminex 96-well plate
- Export optimized data for graphing

TABLE // 07

Quantist Software Minimum Requirements

Processor	3.0 GHz i7 6th gen
Ram	8 GB
Hard Drive	256 GB
Ports	1-USB 1-NIC
OS	Windows 10 Pro 64bit
Screen Resolution	1600x900 24 bit color



Try free for 30 days | bio-techne.com/reagents/luminex/luminex-software-quantist

Custom Services

Accelerate Biomarker Discovery, Validation, and Detection with Unique Analyte Panels

Our R&D Systems development team has decades of experience developing, optimizing, and validating the highest quality Luminex assays for research through to clinical studies and diagnostic use. Our iterative development process includes comprehensive antibody screening, assay optimization, and manufacturability testing. Our dedicated scientists and project managers work closely with you to ensure your goals are met in a timely, efficient manner.

R&D Systems will work closely with you to produce assays that are tailored to your project needs. This may include performance optimization of analytes from our massive menu of analytes, or the creation of an assay for a biomarker that we don't currently offer in either Luminex product category, or even the evaluation of specific sample types that our kits were not initially tested for. We will turn your multiplexing idea into reality by working closely with you to find the best solution for your needs.

Available Services:

- **Bead Region Conflict Resolution:** Is a bead region conflict preventing you from getting all your analytes on the same panel? We can reassign bead regions so you can get all your results with one assay. Bead region change services offered at no extra charge!*
- **Bulk Packaging:** When ordering multiple kits, we can package kit materials in bulk to reduce packaging waste and storage space at no extra charge.*
- **New Analyte Development:** With a fast turnaround, R&D Systems can add new targets to your existing Luminex panel. Using the extensive collection of catalog and non-catalog antibodies and proteins, our teams can quickly assess the feasibility of your target and add it to an existing panel, ultimately reducing your time to data and minimizing the sample volume required.
- **Lot Sequestration:** To reduce potential lot qualifications in a long-term study, we can save you time by reserving your Luminex materials, so everything is built from the same batch.* **Terms and conditions apply.*

Explore Our Custom Luminex Assay Services & Connect with a Specialist

Scan the QR Code or Contact:
rndsystems.com/luminex-services

Ordering R&D Systems Luminex Assays

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

- Save your selected Luminex Assay configuration for easy re-ordering.
- View how the selection of each assay specification changes the final kit options.
- Find pricing options for premixed or user mixed High Performance Assays.
- Alter your assay selections at any time with the tool's easy navigation.
- Get access to ordering or technical support via phone and email.

TABLE // 08

Filter Analytes

✓ MMP-12	FABP4/A-FABP	MMP-13
✓ IL-6	✓ Ferritin	✓ IL-2
Myoglobin	✓ Aggrecan	Nectin-4
✓ FGF-13	✓ TNF-alpha	Nephrin
✓ FGF-23	Neuropilin-1	✓ IL-8/CXCL8
Angiogenin	Fibronectin	✓ Beta-NGF
Angiopoientin-1	✓ NT-3	Angiopoietin

Example of analyte selections to build your custom panel.

Ordering Assays Online is Easy

Scan the QR Code or Contact:
rndsystems.com/luminex

Find Additional Resources at Bio-Techne Academy

The Luminex Learning Plan at the Bio-Techne Academy has all the training you need to get up and running with your Luminex instrument, assays, and Quantist Analysis software. The course includes modules on Luminex technology, installation, ordering and running R&D Systems assays, how to analyze data, instrument maintenance protocols and troubleshooting. Align the team with courses ranging from a quick refresher to new employee training.

bio-techne academy

Self-Register to Get More!

Scan the QR Code or Contact:
academy.bio-techne.com/learn

TABLE // 09

R&D Systems Luminex High Performance Assays Fixed Panels

Human Cytokine Panel 15-Plex, LKTM011B					
IFN-alpha	IL-1 beta/IL-1F2	IL-3	IL-7	IL-15	
IFN-gamma	IL-1ra/IL-1F3	IL-4	IL-9	IL-33	
IL-1 alpha/IL-1F1	IL-2	IL-6	IL-10	VEGF	
Human Chemokine Panel 8-Plex, LKTM012B					
CCL2/JE/ MCP-1	CCL4/MIP-1 beta	CCL11/Eotaxin	CXCL10/IP-10/ CRG-2		
CCL3/MIP-1 alpha	CCL5/RANTES	CXCL1/GRO alpha	IL-8/CXCL8		
Human Growth Factor Panel 21-Plex, LKTM013B					
CCL3/MIP-1 alpha	EGF	Granzyme B	IL-9	PDGF-AA	VEGF
CCL19/MIP-3 beta	FGF basic/FGF2/ bFGF	IFN-beta	IL-17E/IL-25	PDGF-AB/BB	
CD40 Ligand/ TNFSF5	Flt-3 Ligand/FLT3L	IL-3	IL-33	TGF-alpha	
CXCL2/GRO beta	G-CSF	IL-8/CXCL8	PD-L1/B7-H1	TRAIL/TNFSF10	
Human Immunotherapy Panel 25- Plex, LKTM010B					
CCL2/JE/ MCP-1	GM-CSF	IL-1 beta/IL-1F2	IL-8/CXCL8	IL-15	
CCL3/MIP-1 alpha	Granzyme B	IL-1ra/IL-1F3	IL-9	IL-17/IL-17A	
CCL4/MIP-1 beta	IFN-alpha	IL-2	IL-10	IL-33	
CD40 Ligand/ TNFSF5	IFN-gamma	IL-4	IL-12 p70	PD-L1/B7-H1	
CXCL10/IP-10/ CRG-2	IL-1 alpha/IL-1F1	IL-6	IL-13	TNF-alpha	
Human Th1/Th2 Panel 13-Plex, LKTM008B					
GM-CSF	IL-2	IL-6	IL-12 p70	TNF-beta	
IFN-gamma	IL-4	IL-9	IL-13		
IL-1 beta/IL-1F2	IL-5	IL-10	TNF-alpha		
Human Th9/Th17/Th22 Panel 18-Plex, LKTM009B					
CCL20/MIP-3 alpha	IFN-gamma	IL-4	IL-9	IL-13	IL-17E/IL-25
CD40 Ligand/ TNFSF5	IL-1 beta/IL-1F2	IL-5	IL-10	IL-15	IL-33
GM-CSF	IL-2	IL-6	IL-12 p70	IL-17/IL-17A	TNF-alpha

TABLE // 09

R&D Systems Luminex High Performance Assays Fixed Panels Continued

Human TIMP Panel 3-Plex, LKTM003					
TIMP-1	TIMP-2	TIMP-4			
Human XL Cytokine Panel 46-Plex, LKTM014B					
CCL2/JE/ MCP-1	CXCL1/GRO alpha	Granzyme B	IL-3	IL-12 p70	PDGF-AB/BB
CCL3/MIP-1 alpha	CXCL2/GRO beta	IFN-alpha 2/IFNA2	IL-4	IL-13	TGF-alpha
CCL4/MIP-1 beta	CXCL10/IP-10/ CRG-2	IFN-beta	IL-5	IL-15	TNF-alpha
CCL5/RANTES	EGF	IFN-gamma	IL-6	IL-17/IL-17A	TNF-beta
CCL11/Eotaxin	FGF basic/FGF2/ bFGF	IL-1 alpha/IL-1F1	IL-7	IL-17E/IL-25	TRAIL/TNFSF10
CCL19/MIP-3 beta	Flt-3 Ligand/FLT3L	IL-1 beta/IL-1F2	IL-8/CXCL8	IL-33	VEGF
CCL20/MIP-3 alpha	G-CSF	IL-1ra/IL-1F3	IL-9	PD-L1/B7-H1	
CD40 Ligand/ TNFSF5	GM-CSF	IL-2	IL-10	PDGF-AA	
Mouse XL Cytokine Panel 45-Plex, LKTM015					
BAFF/BLyS/ TNFSF13B	CXCL1/KC	I-CAM	IL-5	IL-16	LIX
CCL2/JE/ MCP-1	CXCL10/IP-10/ CRG-2	IFN-gamma	IL-6	IL-17A	M-CSF
CCL3/MIP-1 alpha	EGF	IL- 1 alpha/IL-1F1	IL-7	IL-18	TIMP-1
CCL4/MIP-1 beta	FGF-basic	IL-1 beta/IL-1F2	IL-9	IL-21	TNF-alpha
CCL5/RANTES	Flt-3 ligand	IL-1ra/IL-1F3	IL-10	IL-27	VEGF
CCL11/Eotaxin	G-CSF	IL-2	IL-11	IL-31	
CCL19/ MIP-3beta	GDF-15	IL-3	IL-12 p70	LDL R	
Chitinase 3-like 1	GM-CSF	IL-4	IL-13	LIF	

TABLE // 10

R&D Systems Luminex High Performance Assays Customizable

Human Biomarker Panel A, Premix FCSTM13, User Mix LBAM000					
BAFF/BLyS	CD14	CXCL13/BLC/ BCA-1	IL-2 Ra	TNF RII/ TNFRSF1B	
CCL20/MIP-3 alpha	CD27/TNFRSF7	gp130	CD25/IL-2R alpha		
Human Cytokine Panel A, Premix FCSTM03, User Mix LUHM000					
CCL2/MCP-1	CXCL5/ENA-78	GM-CSF	IL-1ra/IL-1F3	IL-6	Thrombopoietin /Tpo
CCL3/MIP-1 alpha	CXCL8/IL-8	IFN-gamma	IL-2	IL-10	VEGF
CCL4/MIP-1 beta	FGF basic	IL-1 alpha/IL-1F1	IL-4	IL-17	
CCL5/RANTES	G-CSF	IL-1 beta/IL-1F2	IL-5	TNF-alpha	
Human High Sensitivity Cytokine Panel A, Premix FCSTM09, User Mix LHSCM000					
CXCL8/IL-8	IFN-gamma	IL-2	IL-5	IL-10	TNF-alpha
GM-CSF	IL-1 beta/IL-1F2	IL-4	IL-6	IL-12 p70	VEGF
Human High Sensitivity Cytokine Panel B, Premix FCSTM14, User Mix LBHS000					
GM-CSF	IL-2	IL-7	IL-17A	IL-23	IL-36 beta
IFN-g	IL-5	IL-13	IL-17F	IL-31	TNF-alpha
IL-1b	IL-6	IL-15	IL-22	IL-33	
Human Immuno-Oncology Panel Performance Panel, Premix FCSTM24					
CCL17/TARC	CD40/TNFRSF5	CXCL5/ENA-78	LAG-3	PD-L2/B7-DC	
CD27/TNFRSF7	CD25/IL-2R alpha	HVEM/TNFRSF14	OX40 Ligand/ TNFSF4	TIM-3	
CD28	CTLA-4	IFN-gamma	PD-L1/B7-H1		
Human Kidney Biomarker Panel, Premix FCSTM16, User Mix LHK000					
Clusterin	CXCL10/IP-10/ CRG-2	Cystatin C	Lipocalin-2/NGAL	Osteopontin (OPN)	TFF3
Human MMP Panel, Premix FCSTM07, User Mix LMPM000					
MMP-1	MMP-3	MMP-8	MMP-10	MMP-13	
MMP-2	MMP-7	MMP-9	MMP-12	EMMPRIN	
Human Metabolic Panel, Premix FCSTM19					
C-Peptide	Ghrelin	Glucagon	IL-6	Leptin/OB	Peptide YY
CCL2/JE/MCP-1	GLP-1	IFN-gamma	Insulin	Pancreatic Polypeptide/PP	TNF-alpha

TABLE // 10

R&D Systems Luminex High Performance Assays Customizable Continued

Human Obesity Panel, Premix FCSTM08, User Mix LOBM000					
Adiponectin/ Acrp30	CCL2/MCP-1	IL-6	Leptin/OB	Serpin E1/PAI-1	
C-Reactive Protein/CRP	Comp. Factor D/ Adipsin	IL-10	Resistin	TNF-a	
Human Tumor Biomarker Panel, Premix FCSTM25					
AFP	CEACAM-5	Fas Ligand	IL-6	Oseopontin/OPN	Thyroglobulin
CA125	CG beta/HCG beta	FGF basic	IL-8	Prolactin	TNF-alpha
CA15-3	Chromagranin A/ CHGA	Glypican-1	Leptin	PSA Total	VEGF
CA19-9	CYFRA21-1	HE4/WFDC2	MIF	SCF/c-kit ligand	
CD40 Ligand	Fas	HGF	Neuron-specific enolase/NSE	TGF-alpha	
Human XL Cytokine Panel, Premix FCSTM18B, User Mix LUXLM000B					
CCL2/MCP -1	CX3CL1/ Fractalkine	GM-CSF	IL-2	IL-10	PDGF-AA
CCL3/MIP-1 alpha	CXCL1/GRO alpha	Granzyme B	IL-3	IL-12 p70	PDGF-BB
CCL4/MIP-1 beta	CXCL2/GRO beta	IFN- alpha	IL-4	IL-13	TGF- alpha
CCL5/RANTES	CXCL10/IP-10	IFN- beta	IL-5	IL-15	TNF- alpha
CCL11/Eotaxin	EGF	IFN- gamma	IL-6	IL-17A	TNF-beta
CCL19/MIP-3 beta	FGF basic/FGF-2	IL-1 alpha	IL-7	IL-17E/IL-25	TRAIL
CCL20/MIP-3 alpha	Flt-3 Ligand	IL-1 beta	IL-8/CXCL8	IL-33	VEGF
CD40 Ligand	G-CSF	IL-1ra	IL-9	PD-L1/B7-H1	
Multi-species TGF-b Panel, Premix FCSTM17, User Mix LTGM00					
TGF- beta1	TGF- beta2	TGF- beta3			
Mouse XL Cytokine Performance Panel, Premix FCSTM20, User Mix LMXL000					
BAFF/BLyS/ TNSFS13B	CXCL1/KC	I-CAM	IL-5	IL-16	LIX
CCL2/JE/MCP-1	CXCL10/IP-10/ CRG-2	IFN-gamma	IL-6	IL-17A	M-CSF
CCL3/MIP-1 alpha	EGF	IL- 1 alpha/IL-1F1	IL-7	IL-18	TIMP-1
CCL4/MIP-1 beta	FGF-basic	IL-1 beta/IL-1F2	IL-9	IL-21	TNF-alpha
CCL5/RANTES	Flt-3 ligand	IL-1ra/IL-1F3	IL-10	IL-27	VEGF
CCL11/Eotaxin	G-CSF	IL-2	IL-11	IL-31	
CCL19/MIP-3beta	GDF-15	IL-3	IL-12 p70	LDL R	
Chitinase 3-like 1	GM-CSF	IL-4	IL-13	LIF	

TABLE // 10

R&D Systems Luminex High Performance Assays Customizable Continued

Non Human Primate XL Cytokine Panel, Premix FCSTM21, User Mix LNHPXL000					
BDNF	CXCL10/IP-10/ CRG-2	IFN- alpha	IL-6	IL-17/IL-17A	TNF- alpha
CCL2/JE/MCP-1	CXCL11/I-TAC	IFN- beta	IL-7	IL-21	VEGF
CCL5/RANTES	CXCL13/BLC/ BCA-1	IFN gamma	IL-8/CXCL8	MIP-1 beta	
CCL11/Eotaxin	FGF basic/FGF2/ bFGF	IL-1 beta/IL-1F2	IL-10	PD-L1/B7-H1	
CCL20/MIP-3 alpha	G-CSF	IL-2	IL-12 p70	PDGF-AA	
CD40 Ligand/ TNFSF5	GM-CSF	IL-4	IL-13	PDGF-BB	
CXCL2/GRO beta/ MIP-2/CINC-3	Granzyme B	IL-5	IL-15	TGF- alpha	

TABLE // 11

R&D Systems Luminex Discovery Assays

Human Discovery Assay, Catalog # LXSAHM		
4-1BB/TNFRSF9/CD137	Angiopoietin-like Protein 4/ANGPTL4	CA125/MUC16
ADAMTS13	Angiopoietin-like Protein 6/ANGPTL6	CA15-3/MUC-1
Adiponectin/Acrp30	APP	Calbindin D
Aggrecan	APRIL/TNFSF13	Carbonic Anhydrase IX/CA9
AgRP/ART	Atrial Natriuretic Peptide/ANP	Cathepsin S
ALCAM/CD166	B7-H3	CCL1/I-309/TCA-3
Aldehyde Dehydrogenase 1-A1/ ALDH1A1	BAFF/BLyS/TNFSF13B	CCL11/Eotaxin
alpha 1-Microglobulin	BCMA/TNFRSF17	CCL13/MCP-4
alpha 2-Macroglobulin	BDNF	CCL14/HCC-1/HCC-3
alpha-Fetoprotein/AFP	beta 2-Microglobulin	CCL15/MIP-1 delta
alpha-N-acetylglucosaminidase/ NAGLU	beta-NGF	CCL17/TARC
alpha-Synuclein	BMP-10	CCL18/PARC
Angiogenin	BMP-2	CCL19/MIP-3 beta
Angiopoietin-1	BMP-4	CCL2/JE/MCP-1
Angiopoietin-2	BMP-7	CCL20/MIP-3 alpha
Angiopoietin-like Protein 3/ANGPTL3	BMP-9	CCL21/6Ckine

TABLE // 11

R&D Systems Luminex Discovery Assays Human Continued

CCL22/MDC	Complement Factor D/Adipsin	EpCAM/TROP1
CCL23/MPIF-1	Contactin-1	EphA2
CCL24/Eotaxin-2/MPIF-2	C-Peptide	ErbB2/Her2
CCL25/TECK	C-Reactive Protein/CRP	ErbB3/Her3
CCL26/Eotaxin-3	Cripto	E-Selectin/CD62E
CCL27/CTACK	CX3CL1/Fractalkine	FABP4/A-FABP
CCL28	CXCL1/GRO alpha/KC/CINC-1	Fas Ligand/TNFSF6
CCL3/MIP-1 alpha	CXCL10/IP-10/CRG-2	Fas/TNFRSF6/CD95
CCL4/MIP-1 beta	CXCL11/I-TAC	Ferritin
CCL5/RANTES	CXCL13/BLC/BCA-1	Fetuin A/AHSG
CCL7/MCP-3/MARC	CXCL14/BRAK	FGF acidic/FGF1
CCL8/MCP-2	CXCL16	FGF basic/FGF2/bFGF
CD117/c-kit	CXCL2/GRO beta/MIP-2/CINC-3	FGF-13
CD14	CXCL4/PF4	FGF-23
CD163	CXCL5/ENA-78	Fibroblast Activation Protein alpha/FAP
CD23/Fc epsilon RII	CXCL6/GCP-2	Fibronectin
CD25/IL-2R alpha	CXCL7/NAP-2	Flt-3 Ligand/FLT3L
CD27/TNFRSF7	CXCL9/MIG	Follistatin-like 1/FSTL1
CD30/TNFRSF8	Cystatin C	Follistatin-related Gene Protein/FLRG
CD31/PECAM-1	DcR3/TNFRSF6B	Furin
CD40 Ligand/TNFSF5	D-dimer	Galectin-1
CD40/TNFRSF5	Dkk-1	Galectin-3
CD44	DPPIV/CD26	Galectin-3BP/MAC-2BP
CEACAM-1/CD66a	DR3/TNFRSF25	Galectin-9
CEACAM-5/CD66e	EGF	Gas6
Chemerin	EMMPRIN/CD147	G-CSF
Chitinase 3-like 1	Endocan/ESM-1	GDF-15
Coagulation Factor III/Tissue Factor	Endoglin/CD105	GDNF
Coagulation Factor XIV/Protein C	Endostatin	GITR/TNFRSF18
Collagen IV alpha 1	Endothelin-1	GM-CSF
Complement Component C2	Enolase 2/Neuron-specific Enolase	gp130
Complement Component C5a	ENPP-2/Autotaxin	Granzyme A
Complement Component C9	EN-RAGE/S100A12	Granzyme B

TABLE // 11

R&D Systems Luminex Discovery Assays Human Continued

Growth Hormone	IL-1ra/IL-1F3	Lymphotoxin-alpha/TNF-beta
HB-EGF	IL-2	MAdCAM-1
HE4/WFDC2	IL-21	MBL
HGF	IL-23	MCAM/CD146
HGFR/c-MET	IL-27	M-CSF
HMW Adiponectin/Acrp30	IL-28A/IFN-lambda 2	M-CSF R/CD115
HTRA2/Omi	IL-28B/IFN-lambda 3	Mesothelin
ICAM-1/CD54	IL-3	MFG-E8
IFN-alpha	IL-31	MIA
IFN-beta	IL-33	MICA
IFN-gamma	IL-34	Midkine
IFN-gamma R1/CD119	IL-36 beta/IL-1F8	MIF
IGFBP-1	IL-4	MMP-1
IGFBP-2	IL-4R alpha	MMP-12
IGFBP-3	IL-5	MMP-13
IGFBP-4	IL-6	MMP-2
IGFBP-6	IL-6R alpha	MMP-3
IGFBP-rp1/IGFBP-7	IL-7	MMP-7
IL-1 alpha/IL-1F1	IL-8/CXCL8	MMP-8
IL-1 beta/IL-1F2	Insulin	MMP-9
IL-1 RI	ITIH4	MSP/MST1
IL-1 RII	Kallikrein 3/PSA	Myeloperoxidase/MPO
IL-10	Kallikrein 5	Myoglobin
IL-11	Kallikrein 6/Neurosin	N-Cadherin
IL-12 p70	Lactoferrin	NCAM-1/CD56
IL-12/IL-23 p40	LBP	Nectin-4
IL-13	Leptin R	Nephrin
IL-15	Leptin/OB	Neuregulin-1 beta 1/NRG1 beta 1
IL-16	LIF	Neuropilin-1
IL-17/IL-17A	LIGHT/TNFSF14	NT-3
IL-17C	Lipocalin-2/NGAL	NT-4
IL-17E/IL-25	LRG1	Oncostatin M/OSM
IL-18/IL-1F4	L-Selectin/CD62L	Osteoactivin/GPNMB
IL-19	Lumican	Osteopontin/OPN

TABLE // 11

R&D Systems Luminex Discovery Assays Human Continued

Osteoprotegerin/TNFRSF11B	SCGF/CLEC11a	TNF-alpha
Park7/DJ-1	Serpin A10/ZPI	Total Inhibin
PBEF/Visfatin	Serpin A12	TRACP/PAP/ACP5
PDGF-AA	Serpin A4/Kallistatin	TRAIL R2/TNFRSF10B
PDGF-AB	Serpin A7/TBG	TRAIL R3/TNFRSF10C
PDGF-BB	Serpin B3/SCCA1	TRAIL/TNFSF10
PDGF-CC	Serpin C1/Antithrombin-III	TRANCE/TNFSF11/RANK L
PDGF-DD	Serpin E1/PAI-1	TREM-1
PD-L1/B7-H1	Serpin F1/PEDF	Troponin I
Pentraxin 2/SAP	SHBG	TSLP
Pentraxin 3/TSG-14	SLPI	UCH-L1/PGP9.5
Periostin/OSF-2	SOST/Sclerostin	ULBP-1
PLA2G7/PAF-AH/Lp-PLA2	SPARC	ULBP-2/5/6
PlGF	SP-D	ULBP-3
Procalcitonin	ST2/IL-33R	ULBP-4/RAET1E
Pro-Collagen I alpha 1	Stanniocalcin 1/STC-1	uPAR
Progranulin/PGRN	Syndecan-1/CD138	u-Plasminogen Activator (uPA)/Urokinase
Prolactin	Syndecan-4	Uromodulin
Properdin	TACI/TNFRSF13B	Uteroglobin/SCGB1A1
Proprotein Convertase 9/PCSK9	Tau	VAP-1/AOC3
Protein S/PROS1	Tenascin C	VCAM-1/CD106
Proteinase 3/Myeloblastin/PRTN3	TFF3	VEGF
P-Selectin/CD62P	TFPI	VEGF-C
RAGE/AGER	TfR (Transferrin R)	VEGFR1/Flt-1
RBP4/Retinol-Binding Protein 4	TGF-alpha	VEGFR2/KDR/Flk-1
Reg3A	Thrombomodulin/BDCA-3	VEGFR3/Flt-4
Relaxin-2	Thrombopoietin/Tpo	Vitamin D BP
Renin	Thrombospondin-2	vWF-A2
Resistin	Thymidine Kinase 1	
ROBO4	Tie-2	
S100A8	TIM-1/KIM-1/HAVCR	
S100A9	TIMP-1	
S100B	TNF RI/TNFRSF1A	
SCF/c-kit Ligand	TNF RII/TNFRSF1B	

TABLE // 11

R&D Systems Luminex Discovery Assays Mouse

Mouse Discovery Assay, Catalog # LXSAMSM		
Adiponectin/Acrp30	Endoglin/CD105	IL-7
Angiopoietin-2	FABP4/A-FABP	LDLR
BAFF/BLyS/TNFSF13B	Fas Ligand/TNFSF6	LIX
beta-NGF	FGF basic/FGF2/bFGF	M-CSF
C1qR1/CD93	FGF-21	MMP-12
CCL11/Eotaxin	G-CSF	MMP-2
CCL12/MCP-5	GDF-15	MMP-3
CCL19/MIP-3 beta	GM-CSF	MMP-8
CCL2/JE/MCP-1	Granzyme B	MMP-9
CCL20/MIP-3 alpha	Haptoglobin	Nephrin
CCL21/6Ckine	HGF	Oncostatin M/OSM
CCL22/MDC	ICAM-1/CD54	Osteopontin/OPN
CCL3/MIP-1 alpha	IFN-gamma	Osteoprotegerin/TNFRSF11B
CCL4/MIP-1 beta	IGFBP-1	PDGF-AA
CCL5/RANTES	IGFBP-3	PDGF-BB
CCL7/MCP-3/MARC	IGF-I/IGF-1	Periostin/OSF-2
CCL8/MCP-2	IL-1 alpha/IL-1F1	PIGF-2
Chitinase 3-like 1	IL-1 beta/IL-1F2	Podocalyxin
Complement Factor D/Adipsin	IL-10	Prolactin
C-Reactive Protein/CRP	IL-12 p70	Proprotein Convertase 9/PCSK9
CXCL1/GRO alpha/KC/CINC-1	IL-13	P-Selectin/CD62P
CXCL10/IP-10/CRG-2	IL-16	Renin
CXCL12/SDF-1 alpha	IL-17/IL-17A	Resistin
CXCL13/BLC/BCA-1	IL-17E/IL-25	S100A8
CXCL16	IL-2	S100A9
CXCL2/GRO beta/MIP-2/CINC-3	IL-27	Serpin E1/PAI-1
Cystatin C	IL-3	SP-D
Dkk-1	IL-33	Syndecan-1/CD138
DPPIV/CD26	IL-4	Thrombospondin-4
EGF	IL-5	TIM-1/KIM-1/HAVCR
EGFR	IL-6	TIMP-1
EMMPRIN/CD147	IL-6R alpha	TIMP-4

TABLE // 11

R&D Systems Luminex Discovery Assays Mouse Continued

TNF RI/TNFRSF1A	TRANCE/TNFSF11/RANK L	VEGF
TNF RII/TNFRSF1B	TWEAK/TNFSF12	VEGFR2/KDR/Flk-1
TNF-alpha	uPAR	

TABLE // 12

R&D Systems Luminex Discovery Assays Rat

Rat Discovery Assay, Catalog # LXSARM		
CXCL1/GRO alpha/KC/CINC-1	IL-1 alpha/IL-1F1	IL-4
CXCL2/GRO beta/MIP-2/CINC-3	IL-1 beta/IL-1F2	IL-6
CXCL3/GRO gamma/CINC-2/DCIP-1	IL-10	L-Selectin/CD62L
GM-CSF	IL-13	TIMP-1
ICAM-1/CD54	IL-18/IL-1F4	TNF-alpha
IFN-gamma	IL-2	VEGF

TABLE // 13

R&D Systems Luminex Discovery Assays Porcine

Porcine Discovery Assay, Catalog # LXSAPM		
CD31/PECAM-1	IL-10	IL-8/CXCL8
CD34	IL-12	MMP-1
C-Reactive Protein/CRP	IL-18/IL-1F4	PDGF-BB
GM-CSF	IL-1ra/IL-1F3	Serpin E1/PAI-1
IFN-gamma	IL-2	TNF-alpha
IL-1 alpha/IL-1F1	IL-4	
IL-1 beta/IL-1F2	IL-6	

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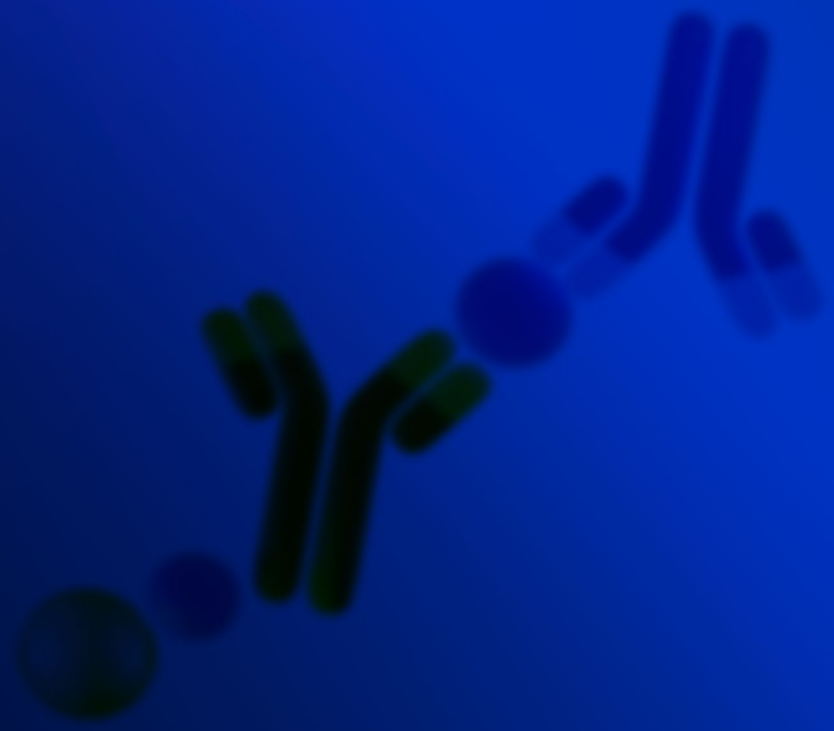

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