

R&D Systems Human Luminex Discovery Assay — Workflow Protocol & Key Benefits

The **R&D Systems™ Human Luminex® Discovery Assay** is designed to streamline multiplex biomarker analysis across a broad range of experimental contexts. By combining premixed reagents with a standardized, plate-based workflow applicable to all plex configurations—from single analytes to panels of up to 50 targets—the assay delivers reproducible performance with minimal hands-on time.

Unlike many competing multiplex platforms that require matrix-specific reagent preparation or protocol adjustments when changing plex size, this assay uses a single, consistent workflow whether you are running a focused cytokine panel or a comprehensive 50-plex screen. This means less time spent on method development and more confidence in cross-study comparisons.

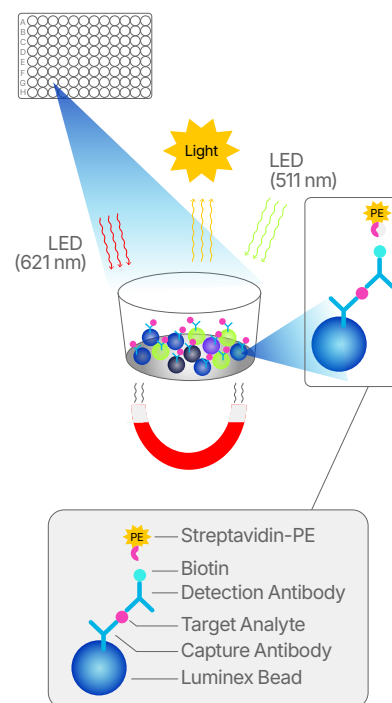
This tech brief describes the assay workflow, highlights key operational advantages, and provides practical guidance for sample handling, reagent preparation, and data analysis.

Principle of the Assay

The Human Luminex Discovery Assay uses magnetic microparticles internally labeled with distinct fluorophore ratios. Each microparticle population is pre-coated with an analyte-specific capture antibody, enabling simultaneous identification and quantification of multiple targets within a single well.

When samples or standards are combined with the microparticle cocktail, target analytes bind to their respective immobilized antibodies. After removal of unbound material, a premixed biotinylated detection antibody cocktail forms a sandwich complex with the captured analytes. Streptavidin-phycoerythrin (Streptavidin-PE) is then added, generating a fluorescent signal proportional to analyte concentration.

Microparticles are read on a Luminex instrument. One light source excites the internal dyes to identify the unique microparticle regions associated with distinct analytes, while a second excites the PE reporter to quantify bound analyte. This dual-signal approach enables robust multiplexing without cross-reactivity between channels.



Sample Collection and Storage

The assay is validated for use with three sample matrices: cell culture supernatants, serum, and plasma (EDTA or heparin anticoagulant). This broad compatibility supports diverse experimental designs without requiring separate optimization for each matrix.

General Collection Guidelines:

- **Cell Culture Supernatants:** Remove particulates by centrifugation prior to use.
- **Serum:** Use a serum separator tube (SST). Allow samples to clot for 30 minutes at room temperature, then centrifuge for 15 minutes at 1,000 × g. Remove serum promptly.
- **Plasma:** Collect using EDTA or heparin anticoagulant. Centrifuge within 30 minutes of collection at 1,000 × g for 15 minutes. Note: citrate plasma has not been validated for this assay.
- **Storage:** All sample types may be analyzed immediately or aliquoted and stored at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Many multiplex platforms require samples to be used immediately after collection. The R&D Systems Human Luminex Discovery Assay supports flexible sample storage at ≤ -20 °C, allowing batching of samples collected across multiple time points without compromising assay performance—an important practical benefit for longitudinal studies and biobanked specimens.

Sample Preparation

All sample types require a minimum of a 2-fold dilution prior to analysis. On the day of the assay, fresh and previously frozen serum and plasma samples must be centrifuged at 16,000 × g for 4 minutes immediately before use or dilution. Use polypropylene tubes for all dilutions.

A standard 2-fold dilution is prepared by combining 75 µL of sample with 75 µL of Calibrator Diluent RD6-52. High-abundance analytes may require larger dilutions using the following recommended volumes:

- **50-fold:** 10 µL sample + 490 µL Calibrator Diluent RD6-52
- **200-fold:** 10 µL sample + 90 µL diluent; then 10 µL of the diluted sample into 190 µL diluent
- **4,000-fold:** 10 µL of 200-fold diluted sample + 190 µL diluent
- **40,000-fold:** 20 µL of 4,000-fold diluted sample + 180 µL diluent

Analyte-specific recommended dilutions are provided in the kit reference table at rndsystems.com/products/lxsahm. These recommendations are a starting point for determining the best sample dilution for your sample set.

Because all sample types—serum, plasma, and cell culture supernatants—are prepared using the same dilution approach, you can process multiple sample matrices within a single run without protocol switching or matrix-specific reagents.

Reagent Preparation

Bring all reagents to room temperature before use. The kit's premixed format dramatically reduces the number of preparation steps compared with assays requiring individual reagent preparation for each analyte.

Wash Buffer

If crystals have formed, warm to room temperature and mix gently until dissolved. Dilute 20 mL of Wash Buffer Concentrate into 480 mL of deionized or distilled water to prepare 500 mL of working wash buffer.

Standards

Reconstitute each unique Standard Cocktail (up to 10 provided, depending on analytes selected) with Calibrator Diluent RD6-52 per the Certificate of Analysis. Allow to sit for a minimum of 15 minutes with gentle agitation before making dilutions. Do not use a rocker or extended vortex. Use polypropylene tubes. Each Standard Cocktail upon reconstitution is a 10× concentrate; combine cocktails into Standard 1 tube to achieve 1× working concentration (see Certificate of Analysis for volumes).

Microparticle Cocktail

Centrifuge the vial for 30 seconds at 1,000 × g, then gently vortex to resuspend. Dilute using Diluent RD2-1 in the mixing bottle provided:

TABLE 1

Wells	Microparticle Cocktail	Diluent RD2-1
96	500 µL	5.00 mL
72	375 µL	3.75 mL
48	250 µL	2.50 mL
24	125 µL	1.25 mL

Table 1. Prepare microparticles within 30 minutes of use. Protect from light. Diluted microparticles cannot be stored.

When CCL5/RANTES is included in the panel, add the CCL5/RANTES microparticle concentrate to the previously diluted microparticle cocktail following the volumes specified in the kit insert.

Biotin-Antibody Cocktail

Centrifuge the vial for 30 seconds at 1,000 × g, then gently vortex. Dilute in Diluent RD2-1 at the same volume ratios as the microparticle cocktail above.

The premixed biotin-antibody cocktail eliminates the need to prepare individual detection antibodies for each analyte—a significant reduction in pipetting steps and a meaningful source of user-to-user variability in competing formats requiring individual antibody preparation.

Streptavidin-PE

Centrifuge the vial for 30 seconds at $1,000 \times g$, then gently vortex. Dilute Streptavidin-PE concentrate in Wash Buffer. Use a polypropylene amber bottle or a polypropylene test tube wrapped in aluminum foil. Protect from light throughout handling and storage.

Assay Procedure

Equilibrate all reagents and samples to room temperature before beginning. Run standards and samples in duplicate for optimal data quality. Protect microparticles and Streptavidin-PE from light at all times.

1. Sample and Standard Addition

Add 50 μL of standards or prepared samples to each well of the microplate.

2. Microparticle Capture Incubation

Resuspend the diluted microparticle cocktail by inversion or vortexing. Add 50 μL to each well and seal the plate with a foil cover. Incubate 2 hours at room temperature on a horizontal orbital shaker (0.12" orbit) at 800 ± 50 rpm. Premixed microparticles enable uniform analyte capture across all targets without any individual bead preparation—simply vortex, add, and incubate.

3. Wash (×3)

Apply a magnetic device to the underside of the microplate. Wait 1 minute, remove liquid, fill each well with 100 μL Wash Buffer, wait 1 minute, then remove liquid. Repeat for a total of three wash cycles. Automated plate washers compatible with magnetic bead assays may also be used to support a faster, more consistent workflow.

Do not blot the plate—this may cause loss of microparticles. Magnetic separation provides efficient, consistent bead retention and low background signal.

4. Detection Antibody Incubation

Add 50 μL of diluted Biotin-Antibody Cocktail to each well. Seal with foil and incubate 1 hour at room temperature at 800 ± 50 rpm. The premixed detection cocktail requires only a single dilution step. Competing assays that supply detection antibodies separately require individual preparation for each analyte, multiplying pipetting steps and the risk of handling variability as panel size increases.

5. Wash (×3)

Repeat the magnetic wash procedure as in Step 3.

6. Streptavidin-PE Incubation

Add 50 μL of diluted Streptavidin-PE to each well. Seal with foil and incubate 30 minutes at room temperature at 800 ± 50 rpm.

7. Final Wash and Resuspension

Repeat the magnetic wash procedure (×3). Add 100 μL of Wash Buffer to each well and incubate for 2 minutes on the shaker at 800 ± 50 rpm to achieve uniform microparticle resuspension.

8. Data Acquisition

Read the plate on a Luminex® or compatible analyzer within 90 minutes.
Resuspend microparticles immediately prior to reading by shaking the plate for 2 minutes at 800 ± 50 rpm.

For full step-by-step protocol guidance, refer to the product insert or [view the instructional assay video at rndsystems.com/resources/protocols/how-run-rd-systems-luminex-assay](https://rndsistemas.com/resources/protocols/how-run-rd-systems-luminex-assay).

Workflow Summary

The complete assay is organized around three core incubations. Hands-on time is limited to brief reagent addition steps, while the majority of assay time is unattended incubation—an approach that supports easy implementation on automated liquid handling platforms already utilized in many customer workflows.

TABLE 2

Step	Action	Duration	Hands-On Time
1	Microparticle Capture Incubation + Wash ×3	2 h	Minimal
2	Biotin-Antibody Incubation + Wash ×3	1 h	Minimal
3	Streptavidin-PE Incubation + Final Wash ×3	30 min	Minimal
Total Assay Time:		~3.5 h	

Data Analysis

Standard concentrations are provided on the Certificate of Analysis; calculate 3-fold dilutions for the remaining levels. Average the replicate readings for each standard and sample and subtract the average blank Median Fluorescence Intensity (MFI).

Create a standard curve for each analyte by reducing the data using computer software, such as R&D Systems Quantist™, capable of generating a five parameter logistic (5-PL) curve-fit. If a 5-PL curve fit is not available use a four parameter logistic (4-PL) curve fit. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

For streamlined data workup, **R&D Systems Quantist™ Data Analysis software** is purpose-built for Luminex data analysis and offers several practical advantages:

- **Intuitive interface:** Minimal learning curve for new users; no scripting or custom macros required.
- **Multi-analyte comparison:** Easily visualize and compare data across analytes and between experimental runs in a single view.
- **Curve flexibility:** Adjustable standard curve parameters allow fine-tuning for analytes with atypical signal behavior.
- **Outlier management:** Easily exclude outliers and low bead counts
- **Comprehensive reporting:** Export data into a comprehensive excel report
- **Side-by-side analysis:** Compare multiple assays at once
- **Multi-standard analysis:** Analyze multiple standard sets in one run
- **Data point exclusion:** Identify and exclude outlier data points without manual recalculation.
- **Broad compatibility:** Accepts data files from all Luminex instrument platforms.

Protocol Advantages at a Glance

Why R&D Systems Human Luminex Discovery Assay?

- **No Overnight Incubations:** Complete the full assay in approximately 3.5 hours—no next-day steps required.
- **Premixed Reagents:** Microparticle and detection antibody cocktails are ready to dilute and use, minimizing pipetting steps and reducing user-to-user variability.
- **Standardized Workflow Across Sample Types:** Serum, plasma, and cell culture supernatants are processed using the same assay workflow and kit reagents—no matrix-specific diluents, media-matching requirements, or protocol changes mid-run.
- **One Protocol, Any Plex Size:** The same workflow applies whether you are running a 1-plex or a 50-plex panel. No reformulation or additional reagents needed when expanding or modifying your panel.
- **All Assay Reagents Included:** Final assay preparation uses the kit wash buffer already integrated into the workflow—eliminating the need for instrument fluids CCS media during assay setup and simplifying automation with liquid handlers and automated washers.
- **Flexible Sample Storage:** Samples may be stored at $\leq -20\text{ }^{\circ}\text{C}$ and used later—ideal for longitudinal studies or multi-site sample collection.

- **Multi-Platform Compatibility:** Compatible with all Luminex instruments, including the MAGPIX®, Luminex® 100/200, FLEXMAP 3D®, xMAP INTELLIFLEX®, and Bio-Rad® Bio-Plex® systems.
- **High Content, Low Volume:** Quantify up to 50 analytes simultaneously from a single 50 µL sample well, conserving precious samples.
- **Streamlined Data Analysis:** Pair with Quantist™ software for automated curve fitting, multi-analyte comparison, and formatted data export.

For additional resources, full protocol documentation, and product ordering information, [visit **rndsystems.com/products/lxsahm**](https://rndsystems.com/products/lxsahm).



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