Magnetic Luminex® Performance Assay

Human Cytokine Premixed Kit A

Catalog Number FCSTM03

For the simultaneous quantitative determination of multiple human cytokine concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

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INTRODUCTION

Cytokines are intercellular signaling proteins released from a wide variety of cells and tissues. They play an integral role in regulating growth and cellular proliferation as well as modulating host response to infection, injury, and inflammation. Cytokines also influence reproduction and bone remodeling. A large number of cytokines are pleiotropic and share similar functions. In addition, many cytokines influence the production of other cytokines. Analysis and quantification of cytokines in biological fluids and cell culture supernates has thus become increasingly important. Methods such as bioassay, enzyme-linked immunosorbent assay (ELISA), intracellular staining, ribonuclease protection assay (RPA) and polymerase chain reaction (PCR) have all been used for quantifying cytokines; however, each of these techniques has limitations associated with it. These techniques are not capable of measuring multiple cytokines simultaneously in a limited sample volume.

This kit can be used to simultaneously assess the levels of multiple cytokines in a single sample. For ease of use, the microparticles and the biotinylated detection antibodies are premixed in respective vials.

Analyte	Microparticle Region	Performance Data Online (www.RnDSystems.com/pdf/...)
CXCL5/ENA-78	12	LUHM254.pdf
FGF basic	13	LUHM233.pdf
G-CSF	14	LUHM214.pdf
GM-CSF	15	LUHM215.pdf
IFN-γ	18	LUHM285.pdf
IL-1α/IL-1F1	19	LUHM200.pdf
IL-1β/IL-1F2	20	LUHM201.pdf
IL-1ra/IL-1F3	21	LUHM280.pdf
IL-2	22	LUHM202.pdf
IL-4	25	LUHM204.pdf
IL-5	26	LUHM205.pdf
IL-6	27	LUHM206.pdf
CXCL8/IL-8	28	LUHM208.pdf
IL-10	29	LUHM217.pdf
IL-17	30	LUHM317.pdf
CCL2/MCP-1	33	LUHM279.pdf
CCL3/MIP-1α	34	LUHM270.pdf
CCL4/MIP-1β	35	LUHM271.pdf
CCL5/RANTES	36	LUHM278.pdf
TNF-α	37	LUHM210.pdf
Tpo	38	LUHM288.pdf
VEGF	39	LUHM293.pdf

PRINCIPLE OF THE ASSAY

Luminex® Performance Assay multiplex kits are designed for use with the Luminex MAGPIX® CCD Imager. Alternatively, kits can be used with the Luminex 100/200™ or Bio-Rad® Bio-Plex®, dual laser, flow-based sorting and detection platforms.

Analyte-specific antibodies are pre-coated onto color-coded magnetic microparticles. Microparticles, standards and samples are pipetted into wells and the immobilized antibodies bind the analytes of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the analytes of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds to the biotinylated antibody, is added to each well. A final wash removes unbound Streptavidin-PE, the microparticles are resuspended in buffer and read using the Luminex MAGPIX Analyzer. A magnet in the analyzer captures and holds the superparamagnetic microparticles in a monolayer. Two spectrally distinct Light Emitting Diodes (LEDs) illuminate the microparticles. One LED identifies the analyte that is being detected and the second LED determines the magnitude of the PE-derived signal, which is in direct proportion to the amount of analyte bound. Each well is imaged with a CCD camera. Kits can also be used with Luminex 100/200 or Bio-Rad Bio-Plex dual laser, flow-based systems.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until these factors have been tested in the Luminex Performance Assay, the possibility of interference cannot be excluded.
- Magnetic Luminex Performance Assays afford the user the benefit of multianalyte analysis of biomarkers in a single complex sample. For each sample type, a single multipurpose diluent is used to optimize recovery, linearity, and reproducibility. Such a multipurpose diluent may not optimize any single analyte to the same degree that a unique diluent selected for analysis of that analyte can optimize conditions. Therefore, some performance characteristics may be more variable than those for assays designed specifically for single analyte analysis.
- **Only the analytes listed on the Standard Value Card can be measured with this kit. Refer to the enclosed certificate of analysis for specific analytes included in this premixed kit.**

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Protect microparticles and Streptavidin-PE from light at all times to prevent photobleaching.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED, DILUTED, OR RECONSTITUTED MATERIAL
Standard Cocktail 1	895531	2 vials of recombinant human cytokines in a buffered protein base with preservatives; lyophilized.	Discard after use. Use fresh standards for each assay.
Standard Cocktail 2	895546	2 vials of recombinant human cytokines in a buffered protein base with preservatives; lyophilized.	
Human Cytokine Premixed Kit A Magnetic Microparticle Cocktail	894537	1.2 mL of a concentrated microparticle cocktail with preservatives.	May be stored for up to 1 month at 2-8 °C.* *Once diluted, any unused microparticle cocktail must be discarded.*
Human Cytokine Premixed Kit A Biotin-Ab Cocktail	894097	1.2 mL of a concentrated biotin antibody cocktail with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Microparticle Diluent	895529	6 mL of a buffered protein base with blue dye and preservative.	
Biotin Antibody Diluent 2	895832	5.5 mL of a buffered protein base with preservative.	
Calibrator Diluent RD5K	895119	21 mL of a 2-fold concentrated solution of a buffered protein base with preservatives. *For cell culture supernate samples. Used diluted 1:2 in this assay.*	
Calibrator Diluent RD6-40	895817	21 mL of a buffered protein base with preservatives. *For serum/plasma samples. May contain a precipitate. Mix well before and during use.*	
Streptavidin-PE	892525	0.07 mL of a 100-fold concentrated streptavidin-phycoerythrin conjugate with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Microplate	641385	1 flat-bottomed 96-well microplate used as a vessel for the assay.	
Mixing Bottles	895505	2 empty 8 mL bottles used for mixing microparticles with Microparticle Diluent.	
Plate Sealers	640445	4 adhesive foil strips.	
Standard Value Card 1	750215	1 card listing the Standard Cocktail 1 reconstitution volume and working standard concentrations for this kit lot.	
Standard Value Card 2	750618	1 card listing the Standard Cocktail 2 reconstitution volume and working standard concentrations for this kit lot.	

*Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Luminex MAGPIX, Luminex 100/200, or Bio-Rad Bio-Plex analyzer with X-Y platform.
- Hand-held microplate magnet or platewasher with a magnetic platform.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, manifold dispenser, or automated dispensing unit.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 800 ± 50 rpm.
- Microcentrifuge.
- **Polypropylene** test tubes for dilution of standards and samples.

PRECAUTION

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION AND STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and 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 before centrifuging 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.

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.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 30 µL of sample + 90 µL of Calibrator Diluent RD6-40. Mix thoroughly.

When assaying CCL5/RANTES, serum and plasma samples must be further diluted 25-fold to a final 100-fold dilution. A suggested 100-fold dilution is 10 µL of the 4-fold diluted sample + 240 µL of Calibrator Diluent RD6-40. Mix thoroughly.

REAGENT PREPARATION

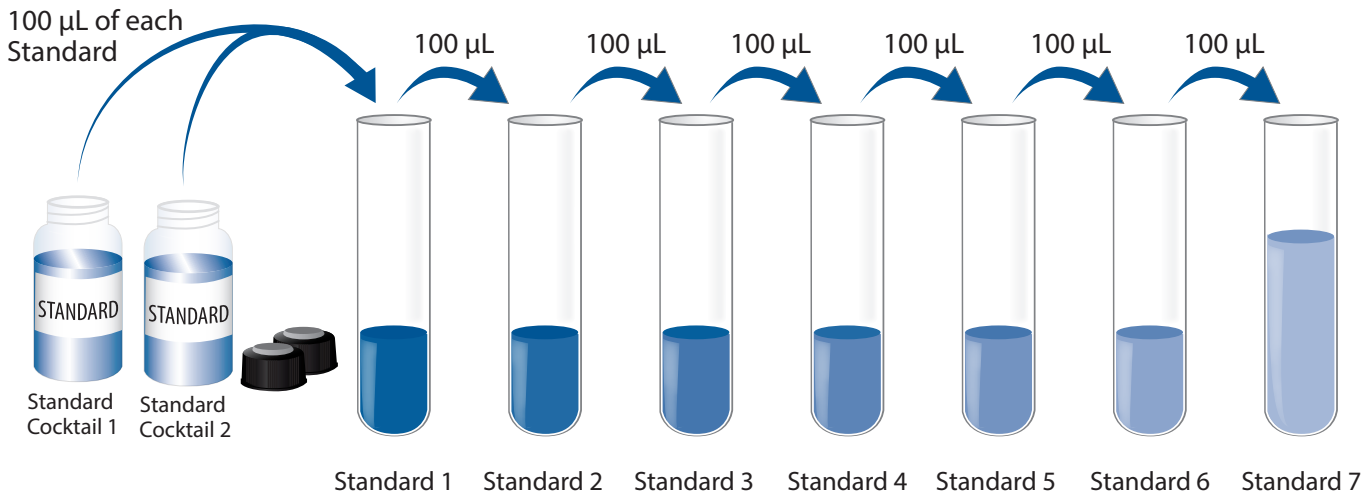
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5K (diluted 1:2) - Add 20 mL of Calibrator Diluent RD5K concentrate to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5K (diluted 1:2).

Standards - Reconstitute one each of Standard Cocktails 1 and 2 with Calibrator Diluent RD5K (diluted 1:2) (*for cell culture supernate samples*) or Calibrator Diluent RD6-40 (*for serum/plasma samples*). Refer to the Standard Value Cards for the reconstitution volumes and the assigned values of working standard 1. Allow the standards to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. This produces a 5X stock of each Standard Cocktail.

Use polypropylene tubes. Pipette 300 µL of the appropriate Calibrator Diluent into a tube labeled working standard 1. Pipette 200 µL of the appropriate Calibrator Diluent into the remaining tubes. Pipette 100 µL of each of the 5X reconstituted Standard Cocktail vials 1 and 2 into the working standard 1 tube. Use working standard 1 to produce a 3-fold dilution series (below). Mix each tube thoroughly before the next transfer. Working standard 1 serves as the high standard. The appropriate Calibrator Diluent serves as the blank.



DILUTED MICROPARTICLE COCKTAIL PREPARATION

1. Centrifuge the Microparticle Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial to resuspend the microparticles, taking precautions not to invert the vial.
3. Dilute the Microparticle Cocktail in the mixing bottle provided.

Number of Wells Used	Microparticle Cocktail	+	Microparticle Diluent
96	1000 µL	+	4.5 mL
72	750 µL	+	3.375 mL
48	500 µL	+	2.25 mL
24	250 µL	+	1.125 mL

Note: *Protect microparticles from light during handling. Diluted microparticles cannot be stored. Prepare microparticles within 30 minutes of use.*

DILUTED BIOTIN ANTIBODY COCKTAIL PREPARATION

1. Centrifuge the Biotin Antibody Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Biotin Antibody Cocktail in Biotin Antibody Diluent 2. Mix gently.

Number of Wells Used	Biotin Antibody Cocktail	+	Biotin Antibody Diluent 2
96	1000 µL	+	4.5 mL
72	750 µL	+	3.375 mL
48	500 µL	+	2.25 mL
24	250 µL	+	1.125 mL

STREPTAVIDIN-PE PREPARATION

Use a polypropylene amber bottle or a polypropylene tube wrapped with aluminum foil. Protect Streptavidin-PE from light during handling and storage.

1. Centrifuge the Streptavidin-PE vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the 100X Streptavidin-PE to a 1X concentration by adding 55 µL of Streptavidin-PE to 5.5 mL of Wash Buffer.

INSTRUMENT SETTINGS

Luminex MAGPIX analyzer:

a) Assign the microparticle region for each analyte being measured (see Introduction on page 1)

b) 50 events/bead

c) Sample size: 50 µL

d) Collect Median Fluorescence Intensity (MFI)

Luminex 100/200 and Bio-Rad Bio-Plex analyzers:

Note: *Calibrate the analyzer using the proper reagents for superparamagnetic microparticles (refer to instrument manual).*

a) Assign the bead region for each analyte being measured (see Introduction on page 1)

b) 50 events/bead

c) Minimum events: 0

d) Flow rate: 60 µL/minute (fast)

e) Sample size: 50 µL

f) Doublet Discriminator gates at approximately 8000 and 16,500

g) Collect MFI

Note: *The CAL2 setting for the Bio-Rad Bio-Plex analyzer should be set at the low RP1 target value.*

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

Note: ***Protect microparticles and Streptavidin-PE from light at all times.***

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Resuspend the diluted Microparticle Cocktail by inversion or vortexing. Add 50 µL of the microparticle mixture to each well of the microplate.
3. Add 50 µL of Standard or sample* per well. Pipette the assay within 15 minutes. Securely cover with a foil plate sealer. Incubate for 3 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 800 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
4. Using a magnetic device designed to accommodate a microplate, wash by applying the magnet to the bottom of the microplate, removing the liquid, filling each well with Wash Buffer (100 µL) and removing the liquid again. Complete removal of liquid is essential for good performance. Perform the wash procedure three times.

   Note: *Refer to the magnetic device user manual for proper wash technique using a round bottom microplate.*
5. Add 50 µL of diluted Biotin-Ab Cocktail to each well. Securely cover with a new foil plate sealer, and incubate for 1 hour at room temperature on the shaker set at 800 ± 50 rpm.
6. Repeat the wash as in step 4.
7. Add 50 µL of diluted Streptavidin-PE to each well. Securely cover with a new foil plate sealer, and incubate for 30 minutes at room temperature on the shaker set at 800 ± 50 rpm.
8. Repeat the wash as in step 4.
9. Resuspend the microparticles by adding 100 µL of Wash Buffer to each well. Incubate for 2 minutes at room temperature on the shaker set at 800 ± 50 rpm.
10. Read within 90 minutes using the Luminex or Bio-Rad analyzer.
    Note: *Resuspend microparticles immediately prior to reading.*

*Serum and plasma samples require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate 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 capable of generating a five parameter logistic (5-PL) curve-fit.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This assay is calibrated against highly purified recombinant human cytokines produced at R&D Systems.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.