Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Magnetic Bead Panel

96-Well Plate Assay

Cat. # PRCYTA-40K, PRCYTA-40K-PX38, PRCYTA-40K-BK38, PRCYTA-40K-PX48, PRCYTA-40K-BK48

MILLIPLEX®

NON-HUMAN PRIMATE CYTOKINE/CHEMOKINE/GROWTH FACTOR PANEL A MAGNETIC BEAD PANEL
96-Well Plate Assay

 # PRCYTA-40K,
 # PRCYTA-40K-PX38, # PRCYTA-40K-BK38,
 # PRCYTA-40K-PX48, # PRCYTA-40K-BK48

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For Research Use Only. Not for Use in Diagnostic Procedures.

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex® Corporation ("Luminex®"), you, the customer, acquire the right under Luminex®'s patent rights, if any, to use this product or any portion of this product, including without limitation the microsphere beads contained herein, only with Luminex®'s laser based fluorescent analytical test instrumentation marketed under the name of Luminex® 100™ IS, 200™, HTS, FLEXMAP 3D®, MAGPIX®.

Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A
Magnetic Bead Panel

INTRODUCTION

"Cytokine" is a general term used for a diverse group of small proteins, peptides or glycoproteins secreted by lymphocytes, monocytes, macrophages and other cells that regulate immune responses, haematopoiesis and lymphocyte development. Cytokines include interleukins, interferons, chemokines and other signaling molecules. Growth factors are extracellular polypeptides that have a positive effect on cell growth and proliferation. They can stimulate growth in a variety of different cell types. Growth factors and cytokines are similar in structure and the way of action. Individual cytokines or growth factors are produced by multiple cell types. This is different from hormones, which tend to be made by specialized glands. Each cytokine or growth factor acts through its own receptor on target cells to generate signaling pathways, and as a consequence to regulate biological processes. Some intracellular signaling components are shared between cytokines and growth factors. Expression of cytokines or growth factors and their receptors is highly regulated. De-regulation may contribute to many diseases such as infectious disease, autoimmune and chronic inflammatory disease, cardiovascular disease, metabolic syndrome, neurological disorders and cancer. Cytokine and growth factor research plays a significant role in achieving a deeper understanding of the immune system and combating related diseases. As the most closely related species to humans, non-human primates are critical models for those studies.

MILLIPLEX® offers the broadest selection of analytes across a wide range of disease states and species. Once the analytes of interest have been identified, you can rely on the quality that we build into each kit to produce results you can trust. In addition to the assay characteristics listed in the protocol, other performance criteria evaluated during the validation process include: cross-reactivity, dilution linearity, kit stability, and sample behavior (e.g. detectability and stability).

Each MILLIPLEX® panel and kit includes:

- Quality controls (QCs) provided to qualify assay performance
- Comparison of standard (calibrator) and QC lots to a reference lot to ensure lot-to-lot consistency
- Optimized serum matrix to mimic native analyte environment
- Detection antibody cocktails designed to yield consistent analyte profiles within panel

In addition each panel and kit meets stringent manufacturing criteria to ensure batch-to-batch reproducibility. The MILLIPLEX® Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Magnetic Bead Panel thus enables you to focus on the therapeutic potential of cytokines and the modulation of cytokine expression. Coupled with the Luminex® xMAP® platform in a magnetic bead format, you receive the advantage of ideal speed and sensitivity, allowing quantitative multiplex detection of dozens of analytes simultaneously, which can dramatically improve productivity.

EMD Millipore's MILLIPLEX® Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Magnetic Bead Panel is part of the most versatile system available for cytokine, chemokine and growth factor research. From our single to multiplex biomarker solutions, we partner with you to design, develop, analytically validate and build the most comprehensive library available for protein detection and quantitation.

- MILLIPLEX® offers you:
  - The ability to select a 38-plex or 48-plex premixed kit.
    - ▪ Note: RANTES is provided as a separate bead vial in both premixed bead kits due to different dilution requirements for serum/plasma samples. For more information, please carefully review the "Sample Collection and Storage" section for serum and plasma samples and "Preparation of Reagents for Immunoassay" for preparation of antibody-immobilized beads when assaying RANTES.
    - ▪ If using tissue/cell culture supernatant samples, the recommended sample dilution is uniform for all analytes. Please review the "Sample Collection and Storage" section for tissue culture supernatant samples.
  - The ability to choose any combination of analytes from our panel of 48 analytes to design a custom kit that better meets your needs.
  - A convenient "all-in-one" box format that gives you the assurance that you will have all the necessary reagents you need to run your assay.

EMD Millipore's MILLIPLEX® Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Magnetic Bead Panel is a 48-plex kit to be used for the simultaneous quantification of any or all of the following analytes in serum or plasma samples and tissue/cell lysate and culture supernatant samples: BCA-1, sCD137, CD40L, Eotaxin, sFASL, FGF-2, Fractalkine, G-CSF, GM-CSF, Granzyme A, Granzyme B, IFNα2, IFNγ, IL-1α, IL-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p70), IL-15, IL-16, IL-17A, IL-17E, IL-18, IL-21, IL-22, IL-23, IL-28A, IL-31, IL-33, IP-10, I-TAC, MCP-1, MIG, MIP-1α, MIP-1β, MIP-3α, Perforin, RANTES, TGFα, TNFα, TNFβ, VEGF-A.

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Please read entire protocol before use.

It is important to use same assay incubation conditions throughout your study.

PRINCIPLE

MILLIPLEX® is based on the Luminex® xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life-sciences and capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded magnetic beads known as MagPlex®-C microspheres.

- Luminex® uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, distinctly colored bead sets of 500 5.6 µm polystyrene microspheres or 80 6.45 µm magnetic microspheres can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- EMD Millipore provides three Luminex® instruments to acquire and analyze data using two detection methods:
    - The Luminex® analyzers Luminex® 200™ and FLEXMAP 3D®, flow cytometry-based instruments that integrate key xMAP® detection components, such as lasers, optics, advanced fluidics and high-speed digital signal processors.
    - The Luminex® analyzer (MAGPIX®), a CCD-based instrument that integrates key xMAP® capture and detection components with the speed and efficiency of magnetic beads.
- Each individual microsphere is identified and the result of its bioassay is quantified based on fluorescent reporter signals. EMD Millipore combines the streamlined data acquisition power of Luminex® xPONENT® acquisition software with sophisticated analysis capabilities of the new MILLIPLEX® Analyst 5.1, integrating data acquisition and analysis seamlessly with all Luminex® instruments.

The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP® technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- For long-term storage, freeze reconstituted standards and controls at ≤ -20°C. Avoid multiple (>2) freeze/thaw cycles.
- **DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibody, and Streptavidin-Phycoerythrin**.

REAGENTS SUPPLIED
Note: Store all reagents at 2 – 8°C

Reagents Supplied	Catalog Number	Volume	Quantity
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Standard	PRCYTA-8040-1 (for configurable 31-plex) or PRCYTA-8040-2 (for 38-plex and 48-plex)	Lyophilized	1 vial
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Quality Controls 1 and 2	PRCYTA-6040-1 (for configurable 31-plex) or PRCYTA-6040-2 (for 38-plex and 48-plex)	Lyophilized	1 vial each
Serum Matrix Note: Contains 0.08% Sodium Azide	MXPRSM-A	Lyophilized	1 vial
Set of one 96-Well Plate with 2 sealers	----------	----------	1 plate 2 sealers
Assay Buffer	L-AB	30 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	60 mL	1 bottle
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Detection Antibodies	PRCYTA-1040-1 (for configurable 31-plex) or PRCYTA-1040-2 (for 38-plex and 48-plex)	3.2 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE4 (Use with Cat# PRCYTA-1040-1) or L-SAPE18 (Use with Cat# PRCYTA-1040-2)	3.2 mL	1 bottle
Bead Diluent (not provided with premixed bead)	LBD	3.5 mL	1 bottle
Mixing Bottle (not provided with premixed panel)	----------	----------	1 bottle

*For details on which reagents ship with which analytes, see table in “Replacement Reagents” section.

REAGENTS SUPPLIED (continued)

Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Antibody-Immobilized Premixed Magnetic Beads:

Premixed 37-plex Beads	PRCYTAPX37-MG	3.5 mL	1 bottle
Premixed 47-plex Beads	PRCYTAPX47-MG	3.5 mL	1 bottle

***Note: RANTES is provided as a separate bead vial in both premixed bead kits due to different dilution requirements for serum/plasma samples. RANTES can only be added in the premix for samples other than serum/plasma. If measuring RANTES in serum/plasma, it is recommended to use a singleplex kit including RANTES only. For more information, please carefully review the sample preparation for serum and plasma samples and the preparation of antibody-immobilized beads sections below when assaying RANTES.**

Included Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Antibody-Immobilized Beads are dependent on customizable selection of analytes within the panel (see below).

Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Antibody-Immobilized Magnetic Beads:

Bead/Analyte Name	Luminex® Magnetic Bead Region	Customizable 48 Analytes (50X concentration, 90 µL) Available	Cat. #	37-Plex Magnetic Premixed Beads	47-Plex Magnetic Premixed Beads
Anti-Human sCD40L Bead	12	✔	HCD40L-MG	✔	✔
Anti-Non-Human Primate BCA-1 Bead	13	✔	PRBCA1-MG		✔
Anti-Human Eotaxin Bead	14	✔	HETXN-MG		✔
Anti-Human FGF-2 Bead	15	✔	HFGF2-MG		✔
Anti-Non-Human Primate sCD137 Bead	18	✔	PRSCD137-MG	✔	✔
Anti-Human Fractalkine Bead	19	✔	HFRACTALKN-MG		✔
Anti-Non-Human Primate IFNγ Bead	20	✔	PRIFNG-MG	✔	✔
Anti-Non-Human Primate IL-16 Bead	21	✔	PRIL16-MG		✔
Anti-Non-Human Primate sFasL Bead	22	✔	PRFASL-MG	✔	✔
Anti-Non-Human Primate G-CSF Bead	25	✔	PRGCSF-MG	✔	✔
Anti-Non-Human Primate GM-CSF Bead	26	✔	PRGMCSF-MG	✔	✔
Anti-Human IL-1α Bead	27	✔	HIL1A-MG		✔
Anti-Non-Human Primate Granzyme A Bead	28	✔	PRGZMA-MG	✔	✔

Bead/Analyte Name (Continued)	Luminex® Magnetic Bead Region	Customizable 48 Analytes (50X concentration, 90 µL) Available	Cat. #	37-Plex Magnetic Premixed Beads	47-Plex Magnetic Premixed Beads
Anti-Non-Human Primate IFNα2 Bead	29	✔	PRIFNA2-MG	✔	✔
Anti-Non-Human Primate IL-10 Bead	30	✔	PRIL10-MG	✔	✔
Anti-Non-Human Primate IL-2 Bead	33	✔	PRIL2-MG	✔	✔
Anti-Non-Human Primate IL-15 Bead	34	✔	PRIL15-MG	✔	✔
Anti-Non-Human Primate IL-17A Bead	35	✔	PRIL17A-MG	✔	✔
Anti-Human IL-6 Bead	36	✔	HIL6-MG	✔	✔
Anti-Non-Human Primate Granzyme B Bead	37	✔	PRGZMB-MG	✔	✔
Anti-Human IL-8 Bead	38	✔	HIL8-MG	✔	✔
Anti-Non-Human Primate IL-1RA Bead	39	✔	PRIL1RA-MG	✔	✔
Anti-Non-Human Primate IL-1β Bead	42	✔	PRIL1B-MG	✔	✔
Anti-Non-Human Primate IL-23 Bead	43	✔	PRIL23-MG	✔	✔
Anti-Human IL-12(p70) Bead	44	✔	HIL12P70-MG	✔	✔
Anti-Non-Human Primate IL-28A Bead	46	✔	PRIL28A-MG		✔
Anti-Non-Human Primate IL-31 Bead	47	✔	PRIL31-MG		✔
Anti-Non-Human Primate IL-33 Bead	48	✔	PRIL33-MG	✔	✔
Anti-Human IL-17E Bead	51	✔	HIL17E-MG		✔
Anti-Non-Human Primate IL-21 Bead	52	✔	PRIL21-MG	✔	✔
Anti-Non-Human Primate IL-4 Bead	53	✔	PRIL4-MG	✔	✔
Anti-Human IL-18 Bead	54	✔	HIL18-MG	✔	✔
Anti-Human IL-22 Bead	55	✔	HIL22-MG	✔	✔
Anti-Non-Human Primate IL-5 Bead	56	✔	PRIL5-MG	✔	✔
Anti-Human IP-10 Bead	57	✔	HIP10-MG	✔	✔
Anti-Human MCP-1 Bead	61	✔	HMCP1-MG	✔	✔
Anti-Non-Human Primate IL-7 Bead	62	✔	PRIL7-MG	✔	✔
Anti-Non-Human Primate I-TAC Bead	63	✔	PRITAC-MG	✔	✔
Anti-Non-Human Primate MIG Bead	64	✔	PRMIG-MG	✔	✔
Anti-Non-Human Primate MIP-1α Bead	65	✔	PRMIP1A-MG	✔	✔

Bead/Analyte Name (Continued)	Luminex® Magnetic Bead Region	Customizable 48 Analytes (50X concentration, 90 µL) Available	Cat. #	37-Plex Magnetic Premixed Beads	47-Plex Magnetic Premixed Beads
Anti-Non-Human Primate MIP-3α Bead	66	✔	PRMIP3A-MG	✔	✔
Anti-Human MIP-1β Bead	67	✔	HMIP1B-MG	✔	✔
Anti-Non-Human Primate TNFα Bead	72	✔	PRTNFA-MG	✔	✔
Anti-Human RANTES Bead	74	✔	HRANTES-MG	*	*
Anti-Human TGFα Bead	75	✔	HTGFA-MG	✔	✔
Anti-Non-Human Primate VEGF-A Bead	76	✔	PRVEGFA-MG	✔	✔
Anti-Human TNFβ Bead	77	✔	HTNFB-MG		✔
Anti-Non-Human Primate Perforin Bead	78	✔	PRPRFN-MG	✔	✔

***Note: RANTES is provided as a separate bead vial in both premixed bead kits due to different dilution requirements for serum/plasma samples. For more information, please carefully review the sample preparation for serum and plasma samples and the preparation of antibody-immobilized beads sections below when assaying RANTES.**

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex® Sheath Fluid (EMD Millipore Catalog # 40-50015) or Luminex® Drive Fluid (EMD Millipore Catalog # MPXDF-4PK)

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
2. Multichannel Pipettes capable of delivering 5 µL to 50 µL or 25 µL to 200 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Aluminum Foil
7. Absorbent Pads
8. Laboratory Vortex Mixer
9. Sonicator (Branson Ultrasonic Cleaner Model # B200 or equivalent)
10. Titer Plate Shaker (VWR® Microplate Shaker Cat # 12620-926 or equivalent)
11. Luminex® 200™, HTS, FLEXMAP 3D®, or MAGPIX® with xPONENT® software by Luminex® Corporation
12. Automatic Plate Washer for magnetic beads (BioTek® 405 LS and 405 TS, EMD Millipore Catalog #40-094, # 40-095, # 40-096, # 40-097 or equivalent) or Handheld Magnetic Separation Block (EMD Millipore Catalog # 40-285 or equivalent).

Note: If a plate washer or handheld magnetic separation block for magnetic beads is not available, one can use a microtiter filter plate (EMD Millipore Catalog # MX-PLATE) to run the assay using a Vacuum Filtration Unit (EMD Millipore Vacuum Manifold Catalog # MSVMHTS00 or equivalent with EMD Millipore Vacuum Pump Catalog # WP6111560 or equivalent).

SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Full Hazard Label

Ingredient, Cat #		Full Label	
10X Wash Buffer	L-WB		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
NHP Cytokine Panel A Std 1 & NHP Cytokine Panel A QC1 & 2 for Std 1, NHP Cytokine Panel A Std 2 & NHP Cytokine Panel A QC1 & 2 for Std 2	PRCYTA-8040-1 & PRCYTA-6040-1, PRCYTA-8040-2 & PRCYTA-6040-2		**Danger**. Harmful if swallowed or if inhaled. Toxic in contact with skin. Causes serious eye damage. May cause damage to Respiratory Tract through prolonged or repeated exposure. May cause damage to Brain through prolonged or repeated exposure if swallowed. Harmful to aquatic life with long lasting effects. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. Take off contaminated clothing and wash before reuse. Store locked up. Dispose of contents/ container to an approved waste disposal plant.

Full Hazard Label Continued

Ingredient, Cat #		Full Label	
NHP Cytokine Panel A Det Abs 1, NHP Cytokine Panel A Det Abs 2	PRCYTA-1040-1, PRCYTA-1040-2		**Warning.** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/ container to an approved waste disposal plant.
Serum Matrix	MXPRSM-A	no symbol required	Harmful to aquatic life with long lasting effects. Avoid release to the environment.
Streptavidin-Phycoerythrin	L-SAPE4		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Streptavidin-Phycoerythrin	L-SAPE18		**Warning.** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/ container to an approved waste disposal plant.

TECHNICAL GUIDELINES

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- The standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at ≤ -20°C for 1 month and at ≤ -80°C for greater than one month.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- Any unused mixed Antibody-Immobilized Beads may be stored in the Mixing Bottle at 2-8°C for up to one month**.**
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes.
- When reading the assay on Luminex® 200™, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended EMD Millipore filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended EMD Millipore filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate using 1 alignment disc.

  For FLEXMAP 3D® when using the solid plate in the kit, the final resuspension should be with 150 µL Sheath Fluid in each well and 75 µL should be aspirated.
- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in background, standard curve and control wells. If

samples are diluted in Assay Buffer, use the Assay Buffer as matrix.

- For serum/plasma samples that require further dilution beyond “Neat”, use the Serum Matrix provided in the kit.
- For cell/tissue homogenate, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Avoid debris, lipids, and cell/tissue aggregates. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.
- Some analytes are temperature sensitive. Please use freshly thawed samples.
- **Note: RANTES is provided as a separate bead vial in both premixed bead kits due to different dilution requirements for serum/plasma samples. RANTES can only be added in the premix for samples other than serum/plasma. If measuring RANTES in serum/plasma, it is recommended to use a singleplex kit including RANTES only. For more information, please carefully review the sample preparation for serum and plasma samples and the preparation of antibody-immobilized beads sections below when assaying RANTES.**
- **Note: The serum/plasma samples of some non-human primate species may require up to 1:100 dilution in Assay Buffer for IL-8.**

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Neat Serum samples (for measuring 47 analytes, not including RANTES) are used. When further dilution is required, use Serum Matrix as the diluent.
- The serum of some non-human primate species may require up to 1:100 dilution in Assay Buffer for IL-8.
- When measuring RANTES in serum, samples should be diluted 1:100 in the Assay Buffer and a standard curve with Assay Buffer should be used accordingly. When further dilution beyond 1:100 is required, use Assay Buffer as the diluent.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.

SAMPLE COLLECTION AND STORAGE (continued)

- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Neat Plasma samples (for measuring 47 analytes, not including RANTES) are used. When further dilution is required, use Serum Matrix as the diluent.
- The plasma of some non-human primate species may require up to 1:100 dilution in Assay Buffer for IL-8.
- When measuring RANTES in plasma, samples should be diluted 1:100 in the Assay Buffer and a standard curve with Assay Buffer should be used accordingly. When further dilution beyond 1:100 is required, use Assay Buffer as the diluent.

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- Tissue culture supernatant may require a dilution with an appropriate control medium prior to assay. Tissue/cell extracts should be done in neutral buffers containing reagents and conditions that do not interfere with assay performance. Excess concentrations of detergent, salt, denaturants, high or low pH, etc. will negatively affect the assay. Organic solvents should be avoided. The tissue/cell extract samples should be free of particles such as cells or tissue debris.

NOTE:

- A maximum of 25 µL per well of diluted or neat sample can be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anti-coagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

If premixed beads are used, sonicate the premixed bead bottle 30 seconds and then vortex for 1 minute before use.
To prepare a 38-Plex premixed beads or a 48-Plex premixed beads, which includes RANTES, add 70 µL of RANTES beads to the 37-Plex premixed bead bottle or the 47-Plex premixed bead bottle, respectively.

(**Note**: Due to high concentration of RANTES in serum/plasma, it has to be measured separately with 1:100 diluted serum/plasma. The 37-Plex premixed beads and the 47-Plex premixed beads are used for measuring all other 37 or 47 analytes, respectively, in serum/plasma with **Neat** serum/plasma. RANTES should only be added to the premix if running samples other than serum/plasma.)

For individual vials of beads, sonicate each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 60 µL from each antibody-bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Bead Diluent. Vortex the mixed beads well. Unused portion may be stored at 2-8°C for up to one month. (Note: Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit.)

Example 1: When using 20 antibody-immobilized beads, add 60 µL from each of the 20 bead vials to the Mixing Bottle. Then add 1.8 mL Bead Diluent .

Example 2: When using 9 antibody-immobilized beads, add 60 µL from each of the 9 bead vials to the Mixing Bottle. Then add 2.46 mL Bead Diluent .

B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL deionized water. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes. Unused portion may be stored at ≤ -20°C for up to one month.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 60 mL of 10X Wash Buffer with 540 mL deionized water. Store the unused portion at 2-8°C for up to one month.

D. Preparation of Serum Matrix

This step is required for serum or plasma samples only.

Add 1 mL deionized water to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 10 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at ≤ -20°C for up to one month.

E. Preparation of Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Standard

1.) Prior to use, reconstitute the Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Standard with 250 µL deionized water. Refer to table below for analyte concentrations. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes. This will be used as Standard 7; the unused portion may be stored at ≤ -20°C for up to one month.

PREPARATION OF REAGENTS FOR IMMUNOASSAY (continued)

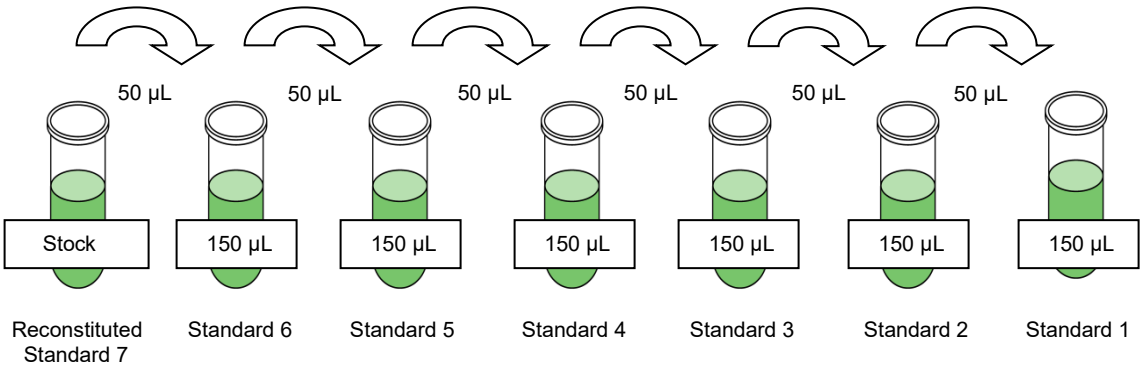
2). Preparation of Working Standards

Label 6 polypropylene microfuge tubes Standard 1 through Standard 6. Add 150 µL of Assay Buffer to each of the 6 tubes. Prepare serial dilutions by adding 50 µL of the reconstituted standard to the Standard 6 tube, mix well and transfer 50 µL of Standard 6 to the Standard 5 tube, mix well and transfer 50 µL of Standard 5 to the Standard 4 tube, mix well and transfer 50 µL of Standard 4 to the Standard 3 tube, mix well and transfer 50 µL of Standard 3 to the Standard 2 tube, mix well and transfer 50 µL of Standard 2 to the Standard 1 tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Standard #	Volume of Deionized Water to Add	Volume of Standard to Add
Standard 7	250 µL	0

Standard #	Volume of Assay Buffer to Add	Volume of Standard to Add
Standard 6	150 µL	50 µL of Standard 7
Standard 5	150 µL	50 µL of Standard 6
Standard 4	150 µL	50 µL of Standard 5
Standard 3	150 µL	50 µL of Standard 4
Standard 2	150 µL	50 µL of Standard 3
Standard 1	150 µL	50 µL of Standard 2

Preparation of Standards



2

Standard	sCD40L (pg/mL)	BCA-1, Eotaxin, GM-CSF, IL-1α, IL-2, IL-17A, IL-6, IP-10, I-TAC, MIP-3α, TNFβ (pg/mL)	FGF-2 (pg/mL)	sCD137, Granzyme B, IL-1β (pg/mL)
Standard 1	48.83	1.22	61.04	0.98
Standard 2	195	4.88	244	3.91
Standard 3	781	20	977	16
Standard 4	3,125	78	3,906	63
Standard 5	12,500	313	15,625	250
Standard 6	50,000	1,250	62,500	1000
Standard 7	200,000	5,000	250,000	4,000

Standard	Fractalkine, IL-17E (pg/mL)	IFNγ, IFNα2, IL-10, IL-22, VEGF-A (pg/mL)	IL-16 (pg/mL)	sFASL, G-CSF, IL-1RA, IL-21, IL-7, MIG, TNFα (pg/mL)
Standard 1	24.41	2.44	36.62	4.88
Standard 2	98	9.77	146	20
Standard 3	391	39	586	78
Standard 4	1,563	156	2,344	313
Standard 5	6,250	625	9,375	1,250
Standard 6	25,000	2,500	37,500	5,000
Standard 7	100,000	10,000	150,000	20,000

Standard	Granzyme A (pg/mL)	IL-15, MIP-1β (pg/mL)	IL-8 (pg/mL)	IL-23, IL-18 (pg/mL)
Standard 1	0.61	6.10	0.05	19.53
Standard 2	2.44	24	0.20	78
Standard 3	9.77	98	0.78	313
Standard 4	39	391	3.13	1,250
Standard 5	156	1,563	13	5,000
Standard 6	625	6,250	50	20,000
Standard 7	2,500	25,000	200	80,000

Standard	IL-12(p70), IL-28A, IL-31, IL-4 (pg/mL)	IL-33 (pg/mL)	IL-5 (pg/mL)	MCP-1 (pg/mL)
Standard 1	12.21	14.65	2.93	3.05
Standard 2	49	59	12	12
Standard 3	195	234	47	49
Standard 4	781	938	188	195
Standard 5	3,125	3,750	750	781
Standard 6	12,500	15,000	3,000	3,125
Standard 7	50,000	60,000	12,000	12,500

Standard	MIP-1α (pg/mL)	RANTES (pg/mL)	TGF-α (pg/mL)	Perforin (pg/mL)
Standard 1	0.73	0.10	0.49	97.66
Standard 2	2.93	0.39	1.95	391
Standard 3	12	1.56	7.81	1,563
Standard 4	47	6.25	31	6,250
Standard 5	188	25	125	25,000
Standard 6	750	100	500	100,000
Standard 7	3,000	400	2,000	400,000

IMMUNOASSAY PROCEDURE

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Standards 0 (Background), Standard 1 through 7, Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.

1. Add 200 µL of Wash Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for 0 pg/mL standard (Background).
4. Add 25 µL of Assay Buffer to the sample wells.
5. Add 25 µL of appropriate matrix solution to the background, standards, and control wells. When assaying serum or plasma, use the Serum Matrix provided in the kit. When assaying tissue culture or other supernatant, use proper control culture medium as the matrix solution. When measuring RANTES in serum or plasma use Assay Buffer as the matrix solution.
6. Add 25 µL of Sample (1:100 dilution for RANTES if using serum or plasma samples, neat for all other analytes) into the appropriate wells.
7. Vortex Mixing Bottle and add 25 µL of the Mixed or Premixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)
8. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker overnight (16-18 hours) at 2-8°C. Alternatively, incubate for 2 hours at room temperature (20-25°C). *An overnight incubation may improve assay sensitivity for some analytes.*

9. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
10. Add 25 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)
11. Seal, cover with foil and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C). **DO NOT ASPIRATE AFTER INCUBATION.**
12. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.
13. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
14. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
15. Add 100 µL of Sheath Fluid (or Drive Fluid if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.
16. Run plate on Luminex® 200™, HTS, FLEXMAP 3D® or MAGPIX® with xPONENT® software.
17. Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating analyte concentrations in samples. (Note: For diluted samples, final sample concentrations should be multiplied by the dilution factor.)

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 25 µL Detection Antibodies per well

Incubate 1 hour at RT

Do Not Aspirate

Add 25 µL Streptavidin-Phycoerythrin per well

Incubate for 30 minutes at RT

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 100 µL Sheath Fluid or Drive Fluid per well

Read on Luminex® (50 µL, 50 beads per bead set)

PLATE WASHING

If using a solid plate, use either a handheld magnet or magnetic plate washer.

A.) Handheld magnet **(EMD Millipore Catalog #40-285)** - Rest plate on magnet for 60 seconds to allow complete settling of magnetic beads. Remove well contents by gently decanting the plate in an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid. Wash plate with 200 µL of Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps as recommended in Assay Procedure.

B.) Magnetic plate washer **(EMD Millipore Catalog #40-094, #40-095, #40-096 and #40-097)** - Please refer to specific automatic plate washer manual for appropriate equipment settings. Please note that after the final aspiration, there will be approximately 25 µL of residual wash buffer in each well. This is expected when using the BioTek® plate washer and this volume does not need to be aspirated from the plate.

If using an automatic plate washer other than BioTek® 405 LS or 405 TS, please refer to the manufacturer's recommendations for programming instructions.

EQUIPMENT SETTINGS

Luminex® 200™, HTS, FLEXMAP 3D®, and MAGPIX® with xPONENT® software:

These specifications are for the Luminex® 200™, Luminex® HTS, Luminex® FLEXMAP 3D®, and Luminex® MAGPIX® with xPONENT® software. Luminex® instruments with other software (e.g. MasterPlex®, StarStation, LiquiChip, Bio-Plex® Manager™, LABScan™100) would need to follow instrument instructions for gate settings and additional specifications from the vendors for reading Luminex® magnetic beads.

For magnetic bead assays, the Luminex® 200™ and HTS instruments must be calibrated with the xPONENT® 3.1 compatible Calibration Kit (EMD Millipore Catalog # LX2R-CAL-K25)) and performance verified with the Performance Verification Kit (EMD Millipore Catalog # LX2R-PVER-K25). The Luminex® FLEXMAP 3D® instrument must be calibrated with the FLEXMAP 3D® Calibrator Kit (EMD Millipore Catalog # F3D-CAL-K25) and performance verified with the FLEXMAP 3D® Performance Verification Kit (EMD Millipore Catalog # F3D-PVER-K25). The Luminex® MAGPIX® instrument must be calibrated with the MAGPIX® Calibration Kit (EMD Millipore Catalog # MPX-CAL-K25) and performance verified with the MAGPIX® Performance Verification Kit (EMD Millipore Catalog # MPX-PVER-K25).

NOTE: When setting up a Protocol using the xPONENT® software, you must select MagPlex® as the Bead Type in the Acquisition settings.

NOTE: These assays cannot be run on any instruments using Luminex® IS 2.3 or Luminex® 1.7 software.

EQUIPMENT SETTINGS (continued)

The Luminex® probe height must be adjusted to the plate provided in the kit. Please use Catalog #MAG-PLATE, if additional plates are required for this purpose.

Events:	50, per bead			
Sample Size:	50 µL			
Gate Settings:	8,000 to 15,000			
Reporter Gain:	Default (low PMT)			
Time Out:	60 seconds			
Bead Set:	Customizable 48-plex Beads			
	sCD40L	12	IL-12(p70)	44
	BCA-1	13	IL-28A	46
	Eotaxin	14	IL-31	47
	FGF-2	15	IL-33	48
	sCD137	18	IL-17E	51
	Fractalkine	19	IL-21	52
	IFNγ	20	IL-4	53
	IL-16	21	IL-18	54
	sFasL	22	IL-22	55
	G-CSF	25	IL-5	56
	GM-CSF	26	IP-10	57
	IL-1α	27	MCP-1	61
	Granzyme A	28	IL-7	62
	IFNα2	29	I-TAC	63
	IL-10	30	MIG	64
	IL-2	33	MIP-1α	65
	IL-15	34	MIP-3α	66
	IL-17A	35	MIP-1β	67
	IL-6	36	TNFα	72
	Granzyme B	37	RANTES	74
	IL-8	38	TGFα	75
	IL-1RA	39	VEGF-A	76
	IL-1β	42	TNFβ	77
	IL-23	43	Perforin	78

QUALITY CONTROLS

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at the EMD Millipore website emdmillipore.com using the catalog number as the keyword.

ASSAY CHARACTERISTICS

Cross-Reactivity

There was no or negligible cross-reactivity between the antibodies for an analyte and any of the other analytes in this panel, except G-CSF had ~5% cross-reactivity with IL-1β.

Detection of Normal Blood Samples From Multiple Non-Human Primate Species[1,2]

Analyte	Rhesus		Cynomolgus		Chimpanzee		Baboon		African Green	
	S (n=10)	P (n=4)	S (n=8)	P (n=4)	S (n=5)	P (n=4)	S (n=5)	P (n=4)	S (n=5)	P (n=4)
sCD40L	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
BCA-1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Eotaxin	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
FGF-2	++	-	++	+	-	-	+	-	-	-
sCD137	++	+	++	++	+++	+++	++	++	++	++
Fractalkine	+++	+++	++	++	+++	++	++	++	+++	+++
IFNγ	++	+	++	-	-	-	++	-	+	++
IL-16	++	-	+	+	+++	++	++	-	+	++
sFasL	++	++	++	++	-	++	+++	+++	++	+++
G-CSF	++	++	++	++	+++	++	++	++	++	++
GM-CSF	++	++	++	++	++	+++	+++	+++	++	++
IL-1α	++	++	++	-	++	++	++	++	++	-
Granzyme A	++	++	++	++	+++	+++	++	+	+	-
IFNα2	++	++	++	+	++	++	++	++	++	+
IL-10	++	++	++	++	+	++	+++	+++	+	-
IL-2	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IL-15	++	-	+	++	+	++	-	+	+	+
IL-17A	++	-	++	++	-	+	+++	+++	+	++
IL-6	+++	+++	+++	++	+++	++	+++	+++	+++	++
Granzyme B	++	-	+	-	++	+++	++	+	-	-
IL-8	+++	+++	+++	+++	+++	++	+++	+++	+++	+++
IL-1RA	++	++	++	+++	+	+++	+++	+++	+	+++
IL-1β	+++	+++	+++	++	+++	+++	+++	+++	+++	+++
IL-23	++	++	++	++	-	++	++	++	++	+
IL-12(p70)	+	-	+	-	-	+	+	-	-	-
IL-28A	++	+++	++	++	++	+++	++	+++	++	++
IL-31	+	-	+	++	+	+	+++	++	+	+
IL-33	+	-	-	++	-	+	++	++	-	-
IL-17E	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IL-21	+++	+++	+++	+++	+++	+++	+++	+++	+++	++
IL-4	++	+	+	+	+	+	+++	++	+	-
IL-18	++	+	-	+	++	+++	-	-	-	-

Analyte	Rhesus		Cynomolgus		Chimpanzee		Baboon		African Green	
	S (n=10)	P (n=4)	S (n=8)	P (n=4)	S (n=5)	P (n=4)	S (n=5)	P (n=4)	S (n=5)	P (n=4)
IL-22	++	+++	++	++	++	++	+++	++	++	++
IL-5	++	+	++	++	-	-	+	++	+	++
IP-10	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
MCP-1	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
IL-7	++	-	++	+	+++	+++	++	+	-	-
I-TAC	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
MIG	++	-	+	+	+++	+++	+++	+++	+	-
MIP-1α	++	+++	+++	++	++	++	+++	+++	++	-
MIP-3a	++	++	++	++	+++	+++	+++	++	++	+++
MIP-1β	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
TNFα	++	++	++	++	+++	++	++	+	++	++
RANTES	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
TGFα	+++	+	+++	++	+++	+++	+++	+++	+++	+++
VEGF-A	++	+	+	+	+	+	++	++	++	+
TNFβ	+++	+++	++	+++	++	+++	+++	+++	++	+++
Perforin	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

1. S=Serum; P=EDTA Plasma.
2. The “+++”, “++”, “+” and “-“ indicate degree of reactivity: “+++” detected all test samples; “++” detected more than one smples but not all; “+” detected one sample; “-“ no samples detected.

ASSAY CHARACTERISTICS (continued)

Assay Sensitivities (minimum detectable concentrations, pg/mL)

Minimum Detectable Concentration (MinDC) is calculated using MILLIPLEX® Analyst 5.1. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions.

Analyte	Overnight Protocol (n = 11 Assays)		2 Hour Protocol (n = 4 Assays)	
	MinDC (pg/mL)	MinDC+2SD (pg/mL)	MinDC (pg/mL)	MinDC+2SD (pg/mL)
sCD40L	16.53	49.95	9.06	19.17
BCA-1	0.37	1.06	0.70	1.75
Eotaxin	1.45	3.21	0.87	1.49
FGF-2	44.87	88.34	39.98	84.88
sCD137	0.31	1.03	0.86	2.53
Fractalkine	7.99	13.42	7.13	12.31
IFNγ	1.37	2.42	1.76	3.76
IL-16	12.31	23.42	10.86	24.43
sFasL	2.51	5.39	2.31	4.72
G-CSF	4.43	8.62	3.82	8.52
GM-CSF	0.23	0.62	0.73	2.72
IL-1α	0.79	2.94	1.73	7.61
Granzyme A	0.27	1.01	0.17	0.59
IFNα2	1.24	2.62	1.39	2.59
IL-10	0.37	0.99	0.39	0.98
IL-2	0.59	1.96	0.31	0.58
IL-15	1.76	4.43	1.41	5.35
IL-17A	0.42	0.88	0.72	1.45
IL-6	0.41	1.03	1.09	2.88
Granzyme B	0.18	0.36	0.46	1.70
IL-8	0.02	0.07	0.15	0.70
IL-1Ra	1.04	2.23	1.22	2.18
IL-1β	0.18	0.37	0.29	0.53
IL-23	4.45	9.78	0.49	1.90
IL-12 (p70)	2.30	4.57	2.57	5.56
IL-28A	10.69	17.72	8.88	17.77
IL-31	2.63	5.63	2.21	6.08
IL-33	5.45	13.09	2.35	4.26
IL-17E	7.26	17.34	5.40	7.75
IL-21	1.33	2.67	0.70	1.78
IL-4	3.27	6.09	3.54	6.83
IL-18	5.20	10.98	8.43	24.14

ASSAY CHARACTERISTICS (continued)

Analyte	Overnight Protocol (n = 11 Assays)		2 Hour Protocol (n = 4 Assays)	
	MinDC (pg/mL)	MinDC+2SD (pg/mL)	MinDC (pg/mL)	MinDC+2SD (pg/mL)
IL-22	2.09	4.88	1.09	2.81
IL-5	0.75	1.62	0.16	0.54
IP-10	0.60	1.13	0.94	2.31
MCP-1	2.71	4.53	2.43	5.18
IL-7	1.10	3.55	3.27	5.98
I-TAC	0.35	0.81	1.08	2.67
MIG	2.39	5.51	1.57	2.44
MIP-1α	0.93	2.09	0.66	1.95
MIP-3α	0.27	0.63	0.39	1.14
MIP-1β	4.28	8.60	3.33	7.96
TNFα	4.19	5.91	2.67	5.29
*RANTES	0.04	0.12	0.14	0.57
TGFα	0.32	1.19	0.08	0.21
VEGF-A	0.50	1.08	0.43	1.62
TNFβ	0.20	0.45	0.29	1.02
Perforin	46.92	73.95	29.22	60.21

*RANTES standard curve with Assay Buffer

ASSAY CHARACTERISTICS (continued)

Precision

Intra-assay precision is generated from the mean of the %CV's from 16 reportable results across two different concentrations of analytes in a single assay. Inter-assay precision is generated from the mean of the %CV's across two different concentrations of analytes across 10 different assays.

Analyte	Overnight Protocol		2 Hour Protocol
	Intra-assay %CV	Inter-assay %CV	Intra-assay %CV
sCD40L	<10	<20	<10
BCA-1	<10	<20	<10
Eotaxin	<10	<20	<10
FGF-2	<10	<20	<10
sCD137	<10	<20	<10
Fractalkine	<10	<20	<10
IFNγ	<10	<20	<10
IL-16	<10	<20	<10
sFasL	<10	<20	<10
G-CSF	<10	<20	<10
GM-CSF	<10	<20	<10
IL-1α	<10	<20	<10
Granzyme A	<10	<20	<10
IFNα2	<10	<20	<10
IL-10	<10	<20	<10
IL-2	<10	<20	<10
IL-15	<10	<20	<10
IL-17A	<10	<20	<10
IL-6	<10	<20	<10
Granzyme B	<10	<20	<10
IL-8	<10	<20	<10
IL-1Ra	<10	<20	<10
IL-1β	<10	<20	<10
IL-23	<10	<20	<10
IL-12 (p70)	<10	<20	<10
IL-28A	<10	<20	<10
IL-31	<10	<20	<10
IL-33	<10	<20	<10
IL-17E	<10	<20	<10
IL-21	<10	<20	<10
IL-4	<10	<20	<10
IL-18	<10	<20	<10
IL-22	<10	<20	<10
IL-5	<10	<20	<10
IP-10	<10	<20	<10
MCP-1	<10	<20	<10

ASSAY CHARACTERISTICS (continued)

Analyte	Overnight Protocol		2 Hour Protocol
	Intra-assay %CV	Inter-assay %CV	Intra-assay %CV
IL-7	<10	<20	<10
I-TAC	<10	<20	<10
MIG	<10	<20	<10
MIP-1α	<10	<20	<10
MIP-3α	<10	<20	<10
MIP-1β	<10	<20	<10
TNFα	<10	<30	<10
*RANTES	<10	<20	<10
TGFα	<10	<20	<10
VEGF-A	<10	<20	<10
TNFβ	<10	<20	<10
Perforin	<10	<20	<10

*RANTES standard curve with Assay Buffer

ASSAY CHARACTERISTICS (continued)

Accuracy

Spike Recovery: The data represent mean percent recovery of spiked standards ranging from low, medium, and high concentration in serum matrices (n=6).

Analyte	Overnight Protocol	2 Hour Protocol
	% Recovery in Serum Matrix	% Recovery in Serum Matrix
sCD40L	84	83
BCA-1	88	92
Eotaxin	86	86
FGF-2	88	88
sCD137	87	90
Fractalkine	83	85
IFNγ	89	86
IL-16	87	85
sFasL	82	85
G-CSF	89	89
GM-CSF	89	91
IL-1α	86	89
Granzyme A	85	85
IFNα2	84	83
IL-10	91	94
IL-2	83	86
IL-15	84	84
IL-17A	85	86
IL-6	83	86
Granzyme B	88	85
IL-8	85	83
IL-1Ra	86	90
IL-1β	80	87
IL-23	93	84
IL-12 (p70)	84	91
IL-28A	83	84
IL-31	92	93
IL-33	88	94
IL-17E	87	84
IL-21	82	87
IL-4	83	86
IL-18	88	90
IL-22	87	88
IL-5	88	84
IP-10	87	83
MCP-1	85	86
IL-7	86	91
I-TAC	87	87

ASSAY CHARACTERISTICS (continued)

Analyte	Overnight Protocol	2 Hour Protocol
	% Recovery in Serum Matrix	% Recovery in Serum Matrix
MIG	86	85
MIP-1α	102	91
MIP-3α	87	84
MIP-1β	85	95
TNFα	86	92
*RANTES	91	90
TGFα	85	88
VEGF-A	85	88
TNFβ	87	84
Perforin	85	86

*RANTES recovery with Assay Buffer

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Insufficient bead count	Plate washer aspirate height set too low	Adjust aspiration height according to manufacturers' instructions.
	Bead mix prepared inappropriately	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting this into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with alcohol flushes, back flushes and washes; or, if needed, probe should be removed and sonicated.
	Probe height not adjusted correctly	When reading the assay on Luminex® 200™, adjust probe height to the kit solid plate using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height to the kit solid plate using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height to the kit solid plate using 1 alignment disc.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and pipetting with multichannel pipettes without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for cross-reacting components (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes.
Beads not in region or gate	Luminex® instrument not calibrated correctly or recently	Calibrate Luminex® instrument based on manufacturer's instructions, at least once a week or if temperature has changed by >3°C.
	Gate settings not adjusted correctly	Some Luminex® instruments (e.g. Bio-Plex®) require different gate settings than those described in the kit protocol. Use instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex® instrument 4 times to rid it of air bubbles, wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.
	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.

Problem	Probable Cause	Solution
Signal for whole plate is same as background	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Streptavidin-Phycoerythrin was not added	Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been removed, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been removed prior to adding Streptavidin-Phycoerythrin	May need to repeat assay if desired sensitivity not achieved.
	Incubations done at inappropriate temperatures, timings or agitation	Assay conditions need to be checked.
Signals too high, standard curves are saturated	Calibration target value set too high	With some Luminex® instruments (e.g. Bio-Plex®) default target setting for RP1 calibrator is set at high PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with standard curve and samples	Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Check with technical support for appropriate protocol modifications.
	Samples contain analyte concentrations higher than highest standard point	Samples may require dilution and reanalysis for just that particular analyte.
	Standard curve was saturated at higher end of curve	See above.
High variation in samples and/or standards	Multichannel pipette may not be calibrated	Calibrate pipettes.
	Plate washing was not uniform	Confirm all reagents are removed completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing.
	Cross-well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipette tips that are used for reagent additions and that pipette tip does not touch reagent in plate.

REPLACEMENT REAGENTS

Reagent	Note	Cat. #
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Standard	For configurable kit	PRCYTA-8040-1
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Standard	For 38- or 48-plex or configurable kit	PRCYTA-8040-2
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Quality Controls 1 and 2	For configurable kit	PRCYTA-6040-1
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Quality Controls 1 and 2	For 38- or 48-plex or configurable kit	PRCYTA-6040-2
Serum Matrix		MXPRSM-A
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Detection Antibodies	For configurable kit	PRCYTA-1040-1
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A Detection Antibodies	For 38- or 48-plex or configurable kit	PRCYTA-1040-2
Streptavidin-Phycoerythrin	For configurable kit	L-SAPE4
Streptavidin-Phycoerythrin	For 38- or 48-plex or configurable kit	L-SAPE18
Assay Buffer		L-AB
Set of two 96-Well plates with sealers		MAG-PLATE
10X Wash Buffer		L-WB
Bead Diluent		LBD
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A 37-Plex Premixed Beads*		PRCYTAPX37-MG
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A 47-Plex Premixed Beads*		PRCYTAPX47-MG
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A 38 Plex Premixed Magnetic Bead Panel		PRCYTA-40K-PX38
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A 48 Plex Premixed Magnetic Bead Panel		PRCYTA-40K-PX48
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A 38 Plex Premixed Magnetic Bead Panel	BULK PACKAGING	PRCYTA-40K-BK38
Non-Human Primate Cytokine/Chemokine/Growth Factor Panel A 48 Plex Premixed Magnetic Bead Panel	BULK PACKAGING	PRCYTA-40K-BK48

*** For individual beads, see below.**

REPLACEMENT REAGENTS (Continued)

Antibody-Immobilized Magnetic Beads

Analyte	***Bead #***	***Cat. #***
sCD40L	12	HCD40L-MG
BCA-1	13	PRBCA1-MG
Eotaxin	14	HETXN-MG
FGF-2	15	HFGF2-MG
sCD137	18	PRSCD137-MG
Fractalkine	19	HFRACTALKN-MG
IFNγ	20	PRIFNG-MG
IL-16	21	PRIL16-MG
sFasL	22	PRFASL-MG
G-CSF	25	PRGCSF-MG
GM-CSF	26	PRGMCSF-MG
IL-1α	27	HIL1A-MG
Granzyme A	28	PRGZMA-MG
IFNα2	29	PRIFNA2-MG
IL-10	30	PRIL10-MG
IL-2	33	PRIL2-MG
IL-15	34	PRIL15-MG
IL-17A	35	PRIL17A-MG
IL-6	36	HIL6-MG
Granzyme B	37	PRGZMB-MG
IL-8	38	HIL8-MG
IL-1RA	39	PRIL1RA-MG
IL-1β	42	PRIL1B-MG
IL-23	43	PRIL23-MG

Analyte	***Bead #***	***Cat. #***
IL-12(p70)	44	HIL12P70-MG
IL-28A	46	PRIL28A-MG
IL-31	47	PRIL31-MG
IL-33	48	PRIL33-MG
IL-17E	51	HIL17E-MG
IL-21	52	PRIL21-MG
IL-4	53	PRIL4-MG
IL-18	54	HIL18-MG
IL-22	55	HIL22-MG
IL-5	56	PRIL5-MG
IP-10	57	HIP10-MG
MCP-1	61	HMCP1-MG
IL-7	62	PRIL7-MG
I-TAC	63	PRITAC-MG
MIG	64	PRMIG-MG
MIP-1α	65	PRMIP1A-MG
MIP-3α	66	PRMIP3A-MG
MIP-1β	67	HMIP1B-MG
TNFα	72	PRTNFA-MG
RANTES	74	HRANTES-MG
TGFα	75	HTGFA-MG
VEGF-A	76	PRVEGFA-MG
TNFβ	77	HTNFB-MG
Perforin	78	PRPRFN-MG

ANALYTE CONTENTS OF SELECT REAGENTS

Analyte/Bead Name	Luminex® Magnetic Bead Region	Customizable 48 Analytes (50X concentration, 90 µL)		PRCYTA PX37-MG (37-Plex Premixed Beads)	PRCYTA PX47-MG (47-Plex Premixed Beads)	PRCYTA-8040-1 (31-plex Standard Mix)	PRCYTA-8040-2 (48-plex Standard Mix)	PRCYTA-1040-1 (31-plex Detection Mix) with L-SAPE4	PRCYTA-1040-2 (48-plex Detection Mix) with L-SAPE-18
		Available	Cat. #						
Anti-H sCD40L	12	✔	HCD40L-MG	✔	✔	✔	✔	✔	✔
Anti-NHP BCA-1	13	✔	PRBCA1-MG		✔		✔		✔
Anti-H Eotaxin	14	✔	HETXN-MG		✔		✔		✔
Anti-H FGF-2	15	✔	HFGF2-MG		✔		✔		✔
Anti-NHP sCD137	18	✔	PRSCD137-MG	✔	✔	✔	✔	✔	✔
Anti-H Fractalkine	19	✔	HFRACTALKN-MG		✔		✔		✔
Anti-NHP IFNγ	20	✔	PRIFNG-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IL-16	21	✔	PRIL16-MG		✔		✔		✔
Anti-NHP sFasL	22	✔	PRFASL-MG	✔	✔	✔	✔	✔	✔
Anti-NHP G-CSF	25	✔	PRGCSF-MG	✔	✔	✔	✔	✔	✔
Anti-NHP GM-CSF	26	✔	PRGMCSF-MG	✔	✔	✔	✔	✔	✔
Anti-Human IL-1α	27	✔	HIL1A-MG		✔		✔		✔
Anti-NHP Granzyme A	28	✔	PRGZMA-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IFNα2	29	✔	PRIFNA2-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IL-10	30	✔	PRIL10-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IL-2	33	✔	PRIL2-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IL-15	34	✔	PRIL15-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IL-17A	35	✔	PRIL17A-MG	✔	✔	✔	✔	✔	✔
Anti-H IL-6	36	✔	HIL6-MG	✔	✔	✔	✔	✔	✔

Analyte/Bead Name	Luminex® Magnetic Bead Region	Customizable 48 Analytes (50X concentration, 90 µL)		PRCYTA PX37-MG (37-Plex Premixed Beads)	PRCYTA PX47-MG (47-Plex Premixed Beads)	PRCYTA-8040-1 (31-plex Standard Mix)	PRCYTA-8040-2 (48-plex Standard Mix)	PRCYTA-1040-1 (31-plex Detection Mix); with L-SAPE4	PRCYTA-1040-2 (48-plex Detection Mix); with L-SAPE-18
		Available	Cat. #						
Anti-NHP Granzyme B	37	✔	PRGZMB-MG	✔	✔	✔	✔	✔	✔
Anti-H IL-8	38	✔	HIL8-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IL-1RA	39	✔	PRIL1RA-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IL-1β	42	✔	PRIL1B-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IL-23	43	✔	PRIL23-MG	✔	✔	✔	✔	✔	✔
Anti-H IL-12(p70)	44	✔	HIL12P70-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IL-28A	46	✔	PRIL28A-MG		✔		✔		✔
Anti-NHP IL-31	47	✔	PRIL31-MG		✔		✔		✔
Anti-NHP IL-33	48	✔	PRIL33-MG	✔	✔		✔		✔
Anti-H IL-17E	51	✔	HIL17E-MG		✔		✔		✔
Anti-NHP IL-21	52	✔	PRIL21-MG	✔	✔		✔		✔
Anti-NHP IL-4	53	✔	PRIL4-MG	✔	✔	✔	✔	✔	✔
Anti-H IL-18	54	✔	HIL18-MG	✔	✔	✔	✔	✔	✔
Anti-H IL-22	55	✔	HIL22-MG	✔	✔		✔		✔
Anti-NHP IL-5	56	✔	PRIL5-MG	✔	✔	✔	✔	✔	✔
Anti-H IP-10	57	✔	HIP10-MG	✔	✔		✔		✔
Anti-H MCP-1	61	✔	HMCP1-MG	✔	✔	✔	✔	✔	✔
Anti-NHP IL-7	62	✔	PRIL7-MG	✔	✔	✔	✔	✔	✔
Anti-NHP I-TAC	63	✔	PRITAC-MG	✔	✔		✔		✔

Analyte/Bead Name	Luminex® Magnetic Bead Region	Customizable 48 Analytes (50X concentration, 90 µL)		PRCYTA PX37-MG (37-Plex Premixed Beads)	PRCYTA PX47-MG (47-Plex Premixed Beads)	PRCYTA-8040-1 (31-plex Standard Mix)	PRCYTA-8040-2 (48-plex Standard Mix)	PRCYTA-1040-1 (31-plex Detection Mix); with L-SAPE4	PRCYTA-1040-2 (48-plex Detection Mix); with L-SAPE-18
		Available	Cat. #						
Anti-NHP MIG	64	✔	PRMIG-MG	✔	✔		✔		✔
Anti-NHP MIP-1α	65	✔	PRMIP1A-MG	✔	✔	✔	✔	✔	✔
Anti-NHP MIP-3α	66	✔	PRMIP3A-MG	✔	✔		✔		✔
Anti-H MIP-1β	67	✔	HMIP1B-MG	✔	✔	✔	✔	✔	✔
Anti-NHP TNFα	72	✔	PRTNFA-MG	✔	✔	✔	✔	✔	✔
Anti-H RANTES	74	✔	HRANTES-MG			✔	✔	✔	✔
Anti-H TGFα	75	✔	HTGFA-MG	✔	✔	✔	✔	✔	✔
Anti-NHP VEGF-A	76	✔	PRVEGFA-MG	✔	✔	✔	✔	✔	✔
Anti-H TNFβ	77	✔	HTNFB-MG		✔		✔		✔
Anti-NHP Perforin	78	✔	PRPRFN-MG	✔	✔	✔	✔	✔	✔

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WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 0 (Background)	Standard 4	QC-1 Control	Etc.								
B	Standard 0 (Background)	Standard 4	QC-1 Control									
C	Standard 1	Standard 5	QC-2 Control									
D	Standard 1	Standard 5	QC-2 Control									
E	Standard 2	Standard 6	Sample 1									
F	Standard 2	Standard 6	Sample 1									
G	Standard 3	Standard 7	Sample 2									
H	Standard 3	Standard 7	Sample 2									