User Guide

MILLIPLEX® Mouse Cytokine/Chemokine/ Growth Factor Expanded Magnetic Bead Panel 1

96-Well Plate Assay

MCYT1-190K, MCYT1-190K-SPX, MCYT1-190K-SPXBK, MCYT1-190K-LPX, and MCYT1-190K-LPXBK

Introduction 3

Principle 5

Storage Conditions Upon Receipt 6

Reagents Supplied 7
- Mouse Cytokine/Chemokine/GF Expansion Panel 1 Antibody-Immobilized Premixed Magnetic Beads 8
- Mouse Cytokine/Chemokine/GF Expansion Panel 1 Antibody-Immobilized Individual Magnetic Beads 9

Materials Required 14
- Reagents 14
- Instrumentation/Materials 14

Safety Precautions 15
- Symbol Definitions 15

Technical Guidelines 18

Sample Collection and Storage 20
- Preparation of Serum Samples 20
- Preparation of Plasma Samples 20
- Preparation of Tissue Culture Supernatant 20

Preparation of Reagents for Immunoassay 21
Preparation of Antibody-Immobilized Beads 21
Preparation of Quality Controls 21
Preparation of Wash Buffer 21
Preparation of Serum Matrix 21
Preparation of Mouse Cytokine/Chemokine/GF Expansion Panel 1 Standard 22
Preparation of Standards 23

Immunoassay Procedure 27

Plate Washing 29
Solid Plate 29

Equipment Settings 30

Quality Controls 33

Assay Characteristics 33
Cross-Reactivity 33
Assay Sensitivities 33
Precision 36
Accuracy 39

Troubleshooting 43

Product Ordering 47

Well Map 55

Notice 56
Technical Assistance 56
Standard Warranty 56
Contact Information 56
Safety Data Sheets (SDS) 56

Introduction

Mouse models are fundamental in the field of biomedical research, serving as essential tools in preclinical and translational studies across various fields such as immunology, cancer, and infectious diseases. Cytokines, chemokines, and growth factors are key mediators of immune system functions capable of signaling through autocrine, paracrine, and endocrine mechanisms. Their pleiotropic immunomodulatory properties allow these biomolecules to react to diverse stimuli and regulate the immune response, either promoting or inhibiting inflammation.

The MILLIPLEX® portfolio 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 verification process include cross-reactivity, dilution linearity, kit stability, and sample behavior (for example 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 kit meets stringent manufacturing criteria to ensure batch-to-batch reproducibility. The MILLIPLEX® Mouse Cytokine/Chemokine/Growth Factor Expanded Panel 1 thus enables you to focus on the modulation of cytokine expression in mouse research models. 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.

The MILLIPLEX® Mouse Cytokine/Chemokine/Growth Factor Expanded Panel 1 is part of the most versatile system available for cytokine and chemokine research. From our single to multiplex biomarker solutions, we partner with you to design, develop, analytically verify and build the most comprehensive library available for protein detection and quantitation.

MILLIPLEX® kits offer you:

- The ability to select a 41-plex or 68-plex premixed option or
- The ability to choose any combination of analytes from our panel of 68 analytes to design a configurable 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.

The MILLIPLEX® Mouse Cytokine/Chemokine/Growth Factor Expanded Panel 1 is a 68-plex kit to be used for the simultaneous quantification of any or all of the following analytes in serum, plasma, or cell culture supernatant samples: BAFF, Betacellulin, CHI3L1, CXCL16, EGF, Eotaxin/CCL11, Erythropoietin, Exodus-2/CCL21/6Ckine, FGF-2, FLT3L, Fractalkine/CX3CL1, G-CSF, GDF-15/MIC-1, GM-CSF, Granzyme B, IFNα, IFNβ, IFNγ, IL-10, IL-11, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-16, IL-17A, IL-17F, IL-18, IL-1α, IL-1β, IL-2, IL–20, IL-22, IL-28B/IFNλ3, IL-3, IL-31, IL-33/NF-HEV (mature), IL-4, IL-5, IL-6, IL-7, IL-9, IP-10/CXCL10, KC/GROα/CXCL1, LIF, LIX/CXCL5, MCP-1/CCL2, MCP-2/CCL8, MCP-3/CCL7, MCP-5/CCL12, M-CSF, MDC/CCL22, MIG/CXCL9, MIP-1α/CCL3, MIP-1β/CCL4, MIP-2/CXCL2, MIP-3α/CCL20, MIP-3β/CCL19, RANTES/CCL5, sCD137/4-1BB/TNFRSF9, sFas/TNFRSF6, sFasL, sICAM-1, sRAGE, TARC/CCL17, TECK/CCL25, TNFα, and VEGF-A.

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

Please read entire protocol before use.

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

Principle

MILLIPLEX® kits are 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® 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.
- The following Luminex® instruments can be used to acquire and analyze data using two detection methods:
- The Luminex® analyzers Luminex® 200™, FLEXMAP 3D®, and xMAP® INTELLIFLEX are 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®, is 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. We combine the streamlined data acquisition power of Luminex® xPONENT® acquisition software with sophisticated analysis capabilities of Belysa® Immunoassay Curve Fitting Software, integrating data acquisition and analysis seamlessly with all Luminex® instruments.
- xMAP® INTELLIFLEX runs on INTELLIFLEX software for instrument control, run setup and generating high quality data with flexible output options. Data can be exported in xPONENT® style CSV files for compatibility with many existing analytical applications, or in the new, customizable INTELLIFLEX file format. The INTELLIFLEX file format is intended for flexibility and simplicity, allowing the user to freely select which data points to include and to reduce the time to analysis.

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

Reagents	Volume	Quantity	Catalogue No.
Mouse Cytokine/Chemokine/GF Expansion Panel 1 Standard	1 vial	Lyophilized	MCYT1-8190-1 (for configurable 35-plex) or MCYT1-8190-2 (for 41-plex and 68-plex)
Mouse Cytokine/Chemokine/GF Expansion Panel 1 Quality Controls 1 and 2	1 vial each	Lyophilized	MCYT1-6190-1 (for configurable 35-plex) or MCYT1-6190-2 (for 41-plex and 68-plex)
Serum Matrix	1 vial	Lyophilized	MXMSM-MCYT1
Set of one 96-Well Plate with 2 sealers	1 plate 2 sealers	-	-
Assay Buffer	1 bottle	30 mL	L-AB
10X Wash Buffer	1 bottle	60 mL	L-WB
Mouse Cytokine/Chemokine/GF Expansion Panel 1 Detection Antibodies	1 bottle	3.2 mL	MCYT1-1190-1 (for configurable 35-plex) or MCYT1-1190-2 (for 41-plex and 68-plex)
Streptavidin-Phycoerythrin	1 bottle	3.2 mL	L-SAPE20
Mixing Bottle *(not provided with premixed panel)*	1 bottle	-	-

Mouse Cytokine/Chemokine/GF Expansion Panel 1 Antibody-Immobilized Premixed Magnetic Beads

Bead/Analyte Name	Volume	Quantity	Catalogue No.
Premixed 41-plex Beads	3.5 mL	1 bottle	MCYT1PMXS-MG
Premixed 68-plex Beads	3.5 mL	1 bottle	MCYT1PMXL-MG

Included Mouse Cytokine/Chemokine/GF Expansion Panel 1 Antibody-Immobilized Beads are dependent on customizable selection of analytes within the panel (see next page).

Mouse Cytokine/Chemokine/GF Expansion Panel 1 Antibody-Immobilized Individual Magnetic Beads

		Configurable 68 Analytes (70X concentration, 70 µL)			
Bead/Analyte Name	**Luminex® Magnetic Bead Region**	**Instrument Compatibility Restrictions**	**41-Plex Magnetic Premixed Beads**	**68-Plex Magnetic Premixed Beads** (not compatible with the MAGPIX® System)	**Catalogue No.**
Anti-Mouse sFasL Bead	9	Not compatible with the MAGPIX® System		✔	MSFASL-MG
Anti-Mouse IL-22 Bead	12	N/A	✔	✔	MIL22-MG
Anti-Mouse G-CSF Bead	13	N/A	✔	✔	MGCSF-MG
Anti-Mouse Eotaxin/CCL11Bead	14	N/A	✔	✔	MCCL11-MG
Anti-Mouse GM-CSF Bead	15	N/A	✔	✔	MGMCSF-MG
Anti-Mouse IL-33 Bead	18	N/A	✔	✔	MIL33-MG
Anti-Mouse IFNγ Bead	19	N/A	✔	✔	MIFNY-MG
Anti-Mouse IL-1α Bead	21	N/A	✔	✔	MIL1A-MG
Anti-Mouse IL-17F Bead	22	N/A	✔	✔	MIL17F-MG
Anti-Mouse IL-1β Bead	25	N/A	✔	✔	MIL1B-MG
Anti- Mouse IL-2 Bead	26	N/A	✔	✔	MIL2-MG
Anti-Mouse IFNβ Bead	27	N/A	✔	✔	MIFNB-MG
Anti-Mouse IL-4 Bead	28	N/A	✔	✔	MIL4-MG
Anti-Mouse IL-3 Bead	29	N/A	✔	✔	MIL3-MG
Anti-Mouse IL-5 Bead	30	N/A	✔	✔	MIL5-MG
Anti-Mouse IL-18 Bead	33	N/A	✔	✔	MIL18-MG
Anti-Mouse IL-6 Bead	34	N/A	✔	✔	MIL6-MG

		Configurable 68 Analytes (70X concentration, 70 µL)			
Bead/Analyte Name	**Luminex® Magnetic Bead Region**	**Instrument Compatibility Restrictions**	**41-Plex Magnetic Premixed Beads**	**68-Plex Magnetic Premixed Beads** (not compatible with the MAGPIX® System)	**Catalogue No.**
Anti-Mouse FGF-2 Bead	35	N/A	✔	✔	MFGF2-MG
Anti-Mouse IL-7 Bead	36	N/A	✔	✔	MIL7-MG
Anti-Mouse EGF Bead	37	N/A	✔	✔	MEGF-MG
Anti-Mouse IL-9 Bead	38	N/A	✔	✔	MIL9-MG
Anti-Mouse IFNα Bead	39	N/A	✔	✔	MIFNA-MG
Anti-Mouse IL-28B/IFNλ3 Bead	42	N/A	✔	✔	MIL28B-MG
Anti-Mouse IL-10 Bead	43	N/A	✔	✔	MIL10-MG
Anti-Mouse MIP-3α Bead	44	N/A		✔	MMIP3A-MG
Anti-Mouse IL-12p40 Bead	45	N/A	✔	✔	MIL12P40-MG
Anti-Mouse Granzyme B Bead	46	N/A		✔	MGZMB-MG
Anti-Mouse IL-12p70 Bead	47	N/A	✔	✔	MIL12P70-MG
Anti-Mouse TARC Bead	48	N/A		✔	MTARC-MG
Anti-Mouse IL-16 Bead	49	Not compatible with the MAGPIX® System		✔	MIL16-MG
Anti-Mouse LIF Bead	51	N/A	✔	✔	MLIF-MG
Anti-Mouse IL-13 Bead	52	N/A	✔	✔	MIL13-MG
Anti-Mouse LIX Bead	53	N/A	✔	✔	MLIX-MG
Anti-Mouse IL-15 Bead	54	N/A	✔	✔	MIL15-MG

		Configurable 68 Analytes (70X concentration, 70 µL)			
Bead/Analyte Name	**Luminex® Magnetic Bead Region**	**Instrument Compatibility Restrictions**	**41-Plex Magnetic Premixed Beads**	**68-Plex Magnetic Premixed Beads** (not compatible with the MAGPIX® System)	**Luminex® Magnetic Bead Region**
Anti-Mouse Exodus-2/ CCL21/6Ckine Bead	55	N/A		✔	MCCL21-MG
Anti-Mouse IL-17A Bead	56	N/A	✔	✔	MIL17A-MG
Anti-Mouse IP-10 Bead	57	N/A	✔	✔	MIP10-MG
Anti-Mouse Fas Bead	58	Not compatible with the MAGPIX® System		✔	MFAS-MG
Anti-Mouse IL–20 Bead	59	Not compatible with the MAGPIX® System		✔	MIL20-MG
Anti-Mouse KC Bead	61	N/A	✔	✔	MKC-MG
Anti-Mouse MCP-1 Bead	62	N/A	✔	✔	MMCP1-MG
Anti-Mouse MIP-3β Bead	63	N/A		✔	MMIP3B-MG
Anti-Mouse MIP-1α Bead	64	N/A	✔	✔	MMIP1A-MG
Anti-Mouse MDC Bead	65	N/A		✔	MMDC-MG
Anti-Mouse MIP-1β Bead	66	N/A	✔	✔	MMIP1B-MG
Anti-Mouse M-CSF Bead	67	N/A	✔	✔	MMCSF-MG
Anti-Mouse sCD137/ 4-1BB/TNFRSF9 Bead	68	Not compatible with the MAGPIX® System		✔	MCD137-MG
Anti-Mouse sICAM-1 Bead	69	Not compatible with the MAGPIX® System		✔	MSICAM1-MG

		Configurable 68 Analytes (70X concentration, 70 µL)			
Bead/Analyte Name	**Luminex® Magnetic Bead Region**	**Instrument Compatibility Restrictions**	**41-Plex Magnetic Premixed Beads**	**68-Plex Magnetic Premixed Beads** (not compatible with the MAGPIX® System)	**Luminex® Magnetic Bead Region**
Anti-Mouse CXCL16 Bead	70	Not compatible with the MAGPIX® System		✔	MCXCL16-MG
Anti-Mouse MCP-5 Bead	72	N/A		✔	MMCP5-MG
Anti-Mouse MIP-2 Bead	73	N/A	✔	✔	MMIP2-MG
Anti-Mouse MIG Bead	74	N/A	✔	✔	MMIG-MG
Anti-Mouse RANTES Bead	75	N/A	✔	✔	MRANTES-MG
Anti-Mouse VEGF-A Bead	76	N/A	✔	✔	MVEGFA-MG
Anti-Mouse TNFα Bead	77	N/A	✔	✔	MTNFA-MG
Anti-Mouse IL-11 Bead	78	N/A		✔	MIL11-MG
Anti-Mouse sRAGE Bead	79	Not compatible with the MAGPIX® System		✔	MSRAGE-MG
Anti-Mouse CCL25/TECK Bead	80	Not compatible with the MAGPIX® System		✔	MCCL25-MG
Anti-Mouse BAFF Bead	81	Not compatible with the MAGPIX® System		✔	MBAFF-MG
Anti-Mouse GDF-15 Bead	82	Not compatible with the MAGPIX® System		✔	MGDF15-MG
Anti-Mouse FLT3L Bead	83	Not compatible with the MAGPIX® System		✔	MFLT3L-MG

		Configurable 68 Analytes (70X concentration, 70 µL)			
Bead/Analyte Name	**Luminex® Magnetic Bead Region**	**Instrument Compatibility Restrictions**	**41-Plex Magnetic Premixed Beads**	**68-Plex Magnetic Premixed Beads** (not compatible with the MAGPIX® System)	**Luminex® Magnetic Bead Region**
Anti-Mouse CHI3L1 Bead	84	Not compatible with the MAGPIX® System		✔	MCHI3L1-MG
Anti-Mouse MCP-2 Bead	85	Not compatible with the MAGPIX® System		✔	MMCP2-MG
Anti-Mouse MCP-3 Bead	86	Not compatible with the MAGPIX® System		✔	MMCP3-MG
Anti-Mouse Erythropoietin Bead	87	Not compatible with the MAGPIX® System		✔	MEPO-MG
Anti-Mouse Fractalkine/CX3CL1 Bead	88	Not compatible with the MAGPIX® System		✔	MCX3CL1-MG
Anti-Mouse IL-31 Bead	89	Not compatible with the MAGPIX® System		✔	MIL31-MG
Anti-Mouse Betacellulin Bead	90	Not compatible with the MAGPIX® System		✔	MBTC-MG

Materials Required (Not provided)

Reagents

MAGPIX® Drive Fluid PLUS (Catalogue No. 40-50030), xMAP® Sheath Fluid PLUS (Catalogue No. 40-50021), or xMAP® Sheath Concentrate PLUS (Catalogue No. 40-50023).

Instrumentation/Materials

- Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
- Multichannel Pipettes capable of delivering 5 µL to 50 µL, or 25 µL to 200 µL
- Reagent Reservoirs
- Polypropylene Microfuge Tubes
- Rubber Bands
- Aluminum Foil
- Absorbent Pads
- Laboratory Vortex Mixer
- Sonicator (Branson Ultrasonic Cleaner Model No. B200 or equivalent)
- Titer Plate Shaker (VWR® Microplate Shaker, Catalogue No. 12620-926 or equivalent)
- Luminex® 200™, FLEXMAP 3D®, MAGPIX® with xPONENT® software or xMAP® INTELLIFLEX with INTELLIFLEX software by Luminex® Corporation.
- Automatic Plate Washer for magnetic beads (BioTek® 405 LS and 405 TS, Catalogue. Nos. 40-094, 40-095, 40-096, 40-097 or equivalent) or Handheld Magnetic Separation Block (Catalogue No. 40-285 or equivalent).

  Note: See Table on pages 8-11 for instrument requirements based on analyte selection.

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.

Symbol Definitions

Ingredient	Catalogue No.	Label	
Mouse Cytokine Expansion Panel 1 Standard 1 Mouse Cytokine Expansion Panel 1 Standard 2	MCYT1-8190-1 MCYT1-8190-2		**Danger.** Harmful if swallowed, in contact with skin or if inhaled. Causes serious eye damage. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. Harmful to aquatic life with long lasting effects. Do not breathe dust. 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/ hearing 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 it before reuse. Dispose of contents/ container to an approved waste disposal plant.

Ingredient	Catalogue No.	Label	
Mouse Cytokine Expansion Panel 1 QC 1 & 2 for Standard 1 Mouse Cytokine Expansion Panel 1 QC 1 & 2 for Standard 2	MCYT1-6190-1 MCYT1-6190-2		**Danger.** Harmful if swallowed, in contact with skin or if inhaled. Causes serious eye damage. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. May cause damage to organs Brain through prolonged or repeated exposure if swallowed. Harmful to aquatic life with long lasting effects. Do not breathe dust. 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/ hearing 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 it before reuse. Dispose of contents/ container to an approved waste disposal plant.
Serum Matrix	MXMSM-MCYT1	No Label Required	Harmful to aquatic life with long lasting effects. Avoid release to the environment. Dispose of contents/ container to an approved waste disposal plant.

Ingredient	Catalogue No.	Label	
Mouse Cytokine Expansion Panel 1 Detection Antibodies 1 Mouse Cytokine Expansion Panel 1 Detection Antibodies 2	MCYT1-1190-1 MCYT1-1190-2		**Warning.** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe mist or vapours. 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.
Streptavidin-Phycoerythrin	L-SAPE20		**Warning.** Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe mist or vapours. 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.
Assay Buffer	L-AB		**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.
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.

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 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 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 PLUS in each well and 75 µL should be aspirated.

For xMAP® INTELLIFLEX, adjust probe height based on the type of plate you are using, place an alignment disk or an alignment sphere in the well according to the protocol recommended by Luminex®.

- 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 1:2, 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.

Sample Collection and Storage

Preparation of Serum Samples

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000 *x g*. 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.
- Serum samples should be diluted 1:2 in the Assay Buffer provided in the kit. For example, in a tube, 30 µL of serum may be combined with 30 µL of Assay Buffer. When further dilution beyond 1:2 is required, use Serum Matrix as the diluent.

Preparation of Plasma Samples

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000 *x g* 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.
- 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.
- Plasma samples should be diluted 1:2 in the Assay Buffer provided in the kit. For example, in a tube, 30 µL of plasma may be combined with 30 µL of Assay Buffer. When further dilution beyond 1:2 is required, use Serum Matrix as the diluent.

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 serum or plasma can be used. Tissue culture or other media may also 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

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.

For **individual vials of beads**, sonicate each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 50 µL from each antibody-bead vial to the Mixing Bottle and bring final volume to 3.5 mL with Assay Buffer. 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 10 antibody-immobilized beads, add 50 µL from each of the 10 bead vials to the Mixing Bottle. Then add 3.0 mL Assay Buffer.

Example 2: When using 35 antibody-immobilized beads, add 50 µL from each of the 35 bead vials to the Mixing Bottle. Then add 1.75 mL Assay Buffer.

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. Transfer the reconstituted Quality Control 1 and Quality Control 2 into two polypropylene microfuge tubes. Unused portion may be stored at ≤ –20 °C for up to one month.

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.

Preparation of Serum Matrix

This step is required for serum or plasma samples only.

Add 2 mL Assay Buffer 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.

Preparation of Mouse Cytokine/Chemokine/GF Expansion Panel 1 Standard

1. Prior to use, reconstitute the Mouse Cytokine/Chemokine/GF Expansion Panel 1 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. Transfer the reconstituted standard to a polypropylene microfuge tube. This will be used as Standard 7; the unused portion may be stored at ≤ –20 °C for up to one month.

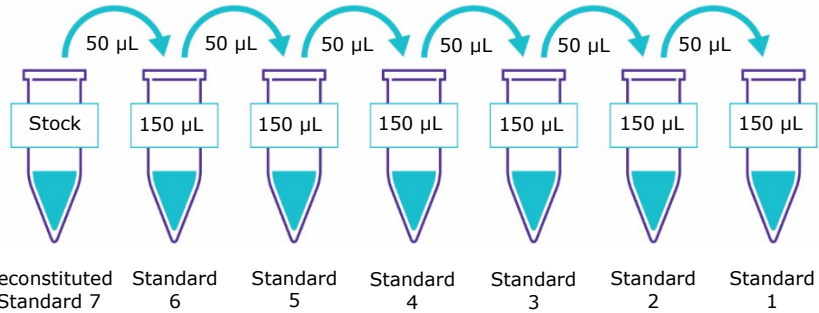
2. Preparation of Working Standards

   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 Standard (Background) will be Assay Buffer.

Standard No.	Add Deionized Water (µL)	Add Standard (volume)
Standard 7	250 µL	0

Standard No.	Add Assay Buffer (µL)	Add Standard (volume)
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



Standard	IL-7 (pg/mL)	MIP-2, MCP-2 (pg/mL)	IL-3 (pg/mL)
Standard 1	0.5	0.6	1.0
Standard 2	2.0	2.4	3.9
Standard 3	7.8	10	16
Standard 4	31	39	63
Standard 5	125	156	250
Standard 6	500	625	1,000
Standard 7	2,000	2,500	4,000

Standard	Fractalkine/CXCL1 (pg/mL)	GDF-15 (pg/mL)	MCP-3 (pg/mL)
Standard 1	1.2	1.5	1.7
Standard 2	4.9	5.9	6.8
Standard 3	20	23	27
Standard 4	78	94	109
Standard 5	313	375	438
Standard 6	1,250	1,500	1,750
Standard 7	5,000	6,000	7,000

Standard	IL-4, MDC (pg/mL)	GM-CSF (pg/mL)	IL-22, IL-17F, IL-2, IL-12p70, IL-17A, MIP-1α, MCP-5, Betacellulin (pg/mL)
Standard 1	1.8	2.2	2.4
Standard 2	7.3	8.8	9.8
Standard 3	29	35	39
Standard 4	117	141	156
Standard 5	469	563	625
Standard 6	1,875	2,250	2,500
Standard 7	7,500	9,000	10,000

Standard	Granzyme B (pg/mL)	IL-28B/IFNλ3, TARC (pg/mL)	Eotaxin/CCL11 (pg/mL)
Standard 1	2.9	3.1	3.2
Standard 2	12	12	13
Standard 3	47	49	51
Standard 4	188	195	203
Standard 5	750	781	813
Standard 6	3,000	3,125	3,250
Standard 7	12,000	12,500	13,000

Standard	IL-1α, IL-5, IFN α, MIP-3 α, IL-13, VEGF-A, TNF α (pg/mL)	IFNβ, IL-9, IL-16, BAFF, FLT3L (pg/mL)	IFNγ (pg/mL)
Standard 1	3.7	4.9	6.1
Standard 2	15	20	24
Standard 3	59	78	98
Standard 4	234	313	391
Standard 5	938	1,250	1,563
Standard 6	3,250	5,000	6,250
Standard 7	15,000	20,000	25,000

Standard	IL-6, IL-12p40, Exodus-2/CCL21/ 6Ckine, RANTES (pg/mL)	LIX, IP-10 (pg/mL)	LIF (pg/mL)
Standard 1	7.3	8.5	9.8
Standard 2	29	34	39
Standard 3	117	137	156
Standard 4	469	547	625
Standard 5	1,875	2,188	2,500
Standard 6	7,500	8,750	10,000
Standard 7	30,000	35,000	40,000

Standard	IL-1β, FGF-2, MCP-1, CXCL16, Erythropoietin (pg/mL)	IL-10, MIP-3β, sCD137/4-1BB/ TNFRSF9, IL-11 (pg/mL)	IL-18, Fas, IL–20, MIP-1β, M-CSF (pg/mL)
Standard 1	12	15	18
Standard 2	49	59	73
Standard 3	195	234	293
Standard 4	781	938	1,172
Standard 5	3,125	3,750	4,688
Standard 6	12,500	15,000	18,750
Standard 7	50,000	60,000	75,000

Standard	MIG (pg/mL)	KC (pg/mL)	IL-33, IL-15, CHI3L1 (pg/mL)
Standard 1	21	22	25
Standard 2	83	88	98
Standard 3	332	352	391
Standard 4	1,328	1,406	1,563
Standard 5	5,313	5,625	6,250
Standard 6	21,250	22,500	25,000
Standard 7	85,000	90,000	100,000

Standard	sFasL (pg/mL)	EGF, sRAGE, CCL25/TECK, IL-31 (pg/mL)	G-CSF (pg/mL)	sICAM-1 (pg/mL)
Standard 1	37	49	98	122
Standard 2	147	195	391	488
Standard 3	586	781	1,563	1,953
Standard 4	2,344	3,125	6,250	7,813
Standard 5	9,375	12,500	25,000	31,250
Standard 6	37,500	50,000	100,000	125,000
Standard 7	150,000	200,000	400,000	500,000

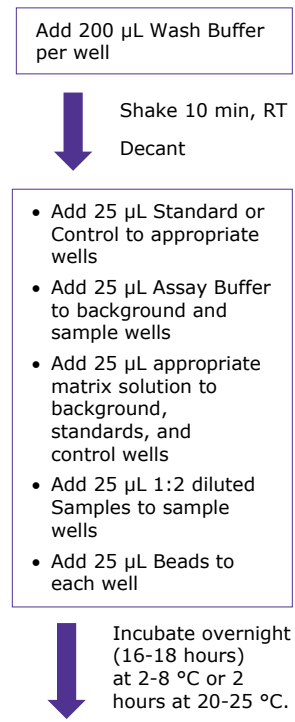
Immunoassay Procedure

- 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. It is recommended to run the assay in duplicate.

  Note: Most instruments will only read the 96-well plate vertically by default.

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 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. When assaying tissue culture or other supernatant, use proper control culture medium as the matrix solution.
6. Add 25 µL of Sample (1:2 diluted or neat) 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).



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).
12. Gently remove well contents and wash plate 3 times following instructions listed in the PLATE WASHING section.
13. Add 25 µL Streptavidin-Phycoerythrin to each well.
14. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25 °C).
15. Gently remove well contents and wash plate 3 times following instructions listed in the PLATE WASHING section.
16. Add 150 µL of Sheath Fluid PLUS (or Drive Fluid PLUS if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.
17. Run plate on Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® with xPONENT® software or xMAP® INTELLIFLEX with INTELLIFLEX Software.
18. 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. For samples diluted as per protocol instructions, multiply by 2. If using another dilution factor, multiple by the appropriate dilution factor.

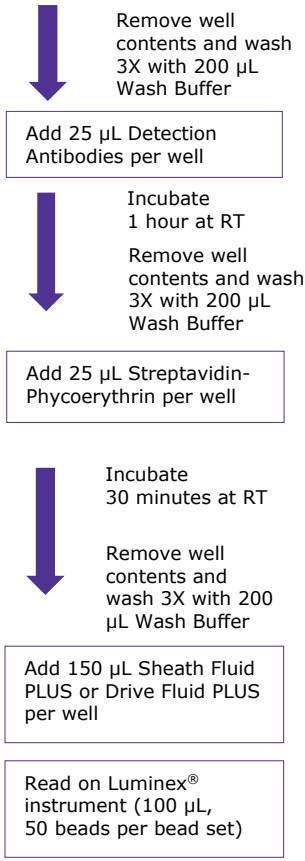


Plate Washing

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

Solid Plate

- Handheld magnet (Catalogue No. 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.

- Magnetic plate washer (Catalogue Nos. 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™, FLEXMAP 3D®, MAGPIX® with xPONENT® software and xMAP® INTELLIFLEX with INTELLIFLEX software:

These specifications are for the above listed instruments and software. Luminex® instruments with other software (for example 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, each instrument must be calibrated, and performance verified with the indicated calibration and verification kits.

Instrument	Calibration Kit	Verification Kit
Luminex® 200™	xPONENT® 3.1 compatible Calibration Kit (Catalogue No. LX2R-CAL-K25)	Performance Verification Kit (Catalogue No. LX2R-PVER-K25)
FLEXMAP 3D®	FLEXMAP 3D® Calibrator Kit (Catalogue No. F3D-CAL-K25)	FLEXMAP 3D® Performance Verification Kit (Catalogue No. F3D-PVER-K25)
xMAP® INTELLIFLEX	xMAP® INTELLIFLEX Calibration Kit (Catalogue No. IFX-CAL-K20)	xMAP® INTELLIFLEX Performance Verification Kit (Catalogue No. IFX-PVER-K20)
MAGPIX®	MAGPIX® Calibration Kit (Catalogue No. MPX-CAL-K25)	MAGPIX® Performance Verification Kit (Catalogue No. 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.

The Luminex® probe height must be adjusted to the plate provided in the kit. Please use Catalogue No. MAG-PLATE, if additional plates are required for this purpose.

Events	50, per bead	
Sample Size	100 µL	
Gate Settings	8,000 to 15,000	
Reporter Gain	Default (low PMT)	
Time Out	60 seconds	
Bead Set	Customizable 68-plex Beads	
	sFasL	9
	IL-22	12
	G-CSF	13
	Eotaxin/CCL11	14
	GM-CSF	15
	IL-33	18
	IFNγ	19
	IL-1α	21
	IL-17F	22
	IL-1β	25
	IL-2	26
	IFNβ	27
	IL-4	28
	IL-3	29
	IL-5	30
	IL-18	33
	IL-6	34
	FGF-2	35
	IL-7	36
	EGF	37
	IL-9	38
	IFNα	39
	IL-28B/IFNλ3	42

IL-10	43
MIP-3α	44
IL-12p40	45
Granzyme B	46
IL-12p70	47
TARC	48
IL-16	49
LIF	51
IL-13	52
LIX	53
IL-15	54
Exodus-2/ CCL21/6Ckine	55
IL-17A	56
IP-10	57
Fas	58
IL–20	59
KC	61
MCP-1	62
MIP-3β	63
MIP-1α	64
MDC	65
MIP-1β	66
M-CSF	67
sCD137/4-1BB/ TNFRSF9	68
sICAM-1	69
CXCL16	70
MCP-5	72
MIP-2	73
MIG	74
RANTES	75

VEGF-A	76
TNFα	77
IL-11	78
sRAGE	79
CCL25/TECK	80
BAFF	81
GDF-15	82
FLT3L	83
CHI3L1	84
MCP-2	85
MCP-3	86
Erythropoietin	87
Fractalkine/CX3CL1	88
IL-31	89
Betacellulin	90

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at our website SigmaAldrich.com using the catalogue 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.

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 = 13 Assays)		2-Hour Protocol (n = 3 Assays)	
	MinDC (pg/mL)	MinDC+2SD (pg/mL)	MinDC (pg/mL)	MinDC+2SD (pg/mL)
sFasL	20.08	29.18	16.87	32.02
IL-22	1.98	2.63	1.36	2.07
G-CSF	43.62	75.60	66.67	174.97
Eotaxin/CCL11	2.75	3.61	2.95	3.38
GM-CSF	0.58	0.84	0.71	1.18
IL-33	11.09	19.25	18.07	43.68
IFNγ	1.46	2.08	1.91	3.08
IL-1α	1.37	2.31	2.46	3.23
IL-17F	0.83	1.49	1.35	2.83
IL-1β	6.61	10.66	4.49	7.74
IL-2	1.19	2.12	0.98	1.72
IFNβ	2.52	4.03	3.39	5.36
IL-4	0.52	0.74	0.42	0.54
IL-3	0.28	0.51	0.47	0.84
IL-5	2.07	4.04	2.15	4.82
IL-18	16.15	23.60	9.10	18.10
IL-6	2.49	4.17	2.85	6.68
FGF-2	18.76	30.75	12.75	37.73
IL-7	0.13	0.19	0.21	0.26
EGF	28.51	43.53	27.16	61.78
IL-9	3.16	6.10	3.63	6.81
IFNα	2.09	3.52	1.33	2.56
IL-28B/IFNλ3	1.61	3.71	2.56	7.15
IL-10	4.88	8.62	6.87	17.67
MIP-3α	3.04	4.97	3.15	5.46
IL-12p40	2.57	4.91	2.58	3.80
Granzyme B	1.08	1.91	1.41	3.91
IL-12p70	0.86	1.44	2.31	5.58

Analyte	Overnight Protocol (n = 13 Assays) MinDC (pg/mL)	Overnight Protocol (n = 13 Assays) MinDC+2SD (pg/mL)	2-Hour Protocol (n = 3 Assays) MinDC (pg/mL)	2-Hour Protocol (n = 3 Assays) MinDC+2SD (pg/mL)
TARC	1.59	2.33	2.07	4.04
IL-16	4.02	5.75	3.59	4.11
LIF	6.29	9.57	6.05	13.37
IL-13	1.52	2.75	2.47	3.94
LIX	6.63	9.81	6.19	6.66
IL-15	12.26	22.18	25.68	43.72
Exodus-2/ CCL21/6Ckine	4.13	6.81	5.70	6.67
IL-17A	0.83	1.40	1.03	2.46
IP-10	6.60	8.26	6.19	9.56
Fas	7.44	12.83	8.71	22.40
IL–20	16.75	23.30	10.61	29.30
KC	12.18	26.87	14.49	29.97
MCP-1	4.98	9.83	5.88	15.25
MIP-3β	14.86	16.16	13.96	16.30
MIP-1α	1.62	2.90	1.44	3.06
MDC	0.97	2.14	0.87	1.70
MIP-1β	17.28	24.90	15.58	22.54
M-CSF	6.06	10.30	5.64	8.41
sCD137/4-1BB/ TNFRSF9	5.42	10.47	6.42	15.08
sICAM-1	78.00	135.14	33.35	63.28
CXCL16	5.91	11.10	5.82	13.15
MCP-5	0.68	1.10	0.81	1.35
MIP-2	0.26	0.57	0.29	0.72
MIG	15.57	24.26	13.97	21.56
RANTES	6.10	9.22	5.29	11.76
VEGF-A	3.12	4.06	2.08	3.56
TNFα	2.07	3.72	1.71	4.29
IL-11	13.06	15.87	9.51	10.25

Analyte	Overnight Protocol (n = 13 Assays) MinDC (pg/mL)	Overnight Protocol (n = 13 Assays) MinDC+2SD (pg/mL)	2-Hour Protocol (n = 3 Assays) MinDC (pg/mL)	2-Hour Protocol (n = 3 Assays) MinDC+2SD (pg/mL)
sRAGE	24.5	49.43	21.82	44.58
CCL25/TECK	15.51	27.79	18.28	30.06
BAFF	1.76	3.66	3.52	10.53
GDF-15	0.55	0.98	0.81	1.84
FLT3L	2.57	4.43	1.23	1.73
CHI3L1	17.23	24.34	7.84	13.30
MCP-2	0.27	0.51	0.32	0.94
MCP-3	1.28	1.71	1.38	1.75
Erythropoietin	8.39	13.77	6.07	15.55
Fractalkine/CX3CL1	0.67	1.24	0.49	0.64
IL-31	32.68	60.12	32.83	76.18
Betacellulin	1.46	2.86	1.95	4.34

Precision

Intra-assay precision is generated from the mean of the % CVs from 8 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 8 different assays for overnight and 3 different assays for same day (see next page).

Analyte	Overnight Protocol		Same Day Protocol	
	Inter-assay %CV	Intra-assay %CV	Inter-assay %CV	Intra-assay %CV
sFasL	<15%	<10%	<15%	<10%
IL-22	<15%	<10%	<15%	<10%
G-CSF	<15%	<10%	<15%	<10%
Eotaxin/CCL11	<15%	<10%	<15%	<10%
GM-CSF	<15%	<10%	<15%	<10%
IL-33	<15%	<10%	<15%	<10%
IFNγ	<15%	<10%	<15%	<10%
IL-1α	<15%	<10%	<15%	<10%
IL-17F	<15%	<10%	<15%	<10%
IL-1β	<15%	<10%	<15%	<10%
IL-2	<15%	<10%	<15%	<10%
IFNβ	<15%	<10%	<15%	<10%
IL-4	<15%	<10%	<15%	<10%
IL-3	<15%	<10%	<15%	<10%
IL-5	<15%	<10%	<15%	<10%
IL-18	<15%	<10%	<15%	<10%
IL-6	<15%	<10%	<15%	<10%
FGF-2	<15%	<10%	<15%	<10%
IL-7	<15%	<10%	<15%	<10%
EGF	<15%	<10%	<15%	<10%
IL-9	<15%	<10%	<15%	<10%
IFNα	<15%	<10%	<15%	<10%
IL-28B/IFNλ3	<15%	<10%	<15%	<10%
IL-10	<15%	<10%	<15%	<10%
MIP-3α	<15%	<10%	<15%	<10%
IL-12p40	<15%	<10%	<15%	<10%
Granzyme B	<15%	<10%	<15%	<10%
IL-12p70	<15%	<10%	<15%	<10%

	Overnight Protocol		Same Day Protocol	
Analyte	**Inter-assay %CV**	**Intra-assay %CV**	**Inter-assay %CV**	**Intra-assay %CV**
TARC	<15%	<10%	<15%	<10%
IL-16	<15%	<10%	<15%	<10%
LIF	<15%	<10%	<15%	<10%
IL-13	<15%	<10%	<15%	<10%
LIX	<15%	<10%	<15%	<10%
IL-15	<15%	<10%	<15%	<10%
Exodus-2/ CCL21/6Ckine	<20%	<10%	<20%	<10%
IL-17A	<15%	<10%	<15%	<10%
IP-10	<15%	<10%	<15%	<10%
Fas	<15%	<10%	<15%	<10%
IL–20	<15%	<10%	<15%	<10%
KC	<15%	<10%	<15%	<10%
MCP-1	<15%	<10%	<15%	<10%
MIP-3β	<15%	<10%	<15%	<10%
MIP-1α	<15%	<10%	<15%	<10%
MDC	<15%	<10%	<15%	<10%
MIP-1β	<15%	<10%	<15%	<10%
M-CSF	<15%	<10%	<15%	<10%
sCD137/4-1BB/ TNFRSF9	<15%	<10%	<15%	<10%
sICAM-1	<15%	<10%	<15%	<10%
CXCL16	<15%	<10%	<15%	<10%
MCP-5	<15%	<10%	<15%	<10%
MIP-2	<15%	<10%	<15%	<10%
MIG	<15%	<10%	<15%	<10%
RANTES	<15%	<10%	<15%	<10%
VEGF-A	<15%	<10%	<15%	<10%
TNFα	<15%	<10%	<15%	<10%

	Overnight Protocol		Same Day Protocol	
Analyte	**Inter-assay %CV**	**Intra-assay %CV**	**Inter-assay %CV**	**Intra-assay %CV**
IL-11	<15%	<10%	<15%	<10%
sRAGE	<15%	<10%	<15%	<10%
CCL25/TECK	<15%	<10%	<15%	<10%
BAFF	<15%	<10%	<15%	<10%
GDF-15	<15%	<10%	<15%	<10%
FLT3L	<15%	<10%	<15%	<10%
CHI3L1	<15%	<10%	<15%	<10%
MCP-2	<15%	<10%	<15%	<10%
MCP-3	<15%	<10%	<15%	<10%
Erythropoietin	<15%	<10%	<15%	<10%
Fractalkine/CX3CL1	<15%	<10%	<15%	<10%
IL-31	<15%	<10%	<15%	<10%
Betacellulin	<15%	<10%	<15%	<10%

Accuracy

Spike Recovery: The data represent mean percent recovery of spiked standards ranging from low, medium, and high concentration in serum matrices (n = 5, see next page).

Analyte	Overnight Protocol % Recovery in Serum Matrix	2-Hour Protocol % Recovery in Serum Matrix
sFasL	94	103
IL-22	90	96
G-CSF	90	110
Eotaxin/CCL11	88	100
GM-CSF	98	97
IL-33	93	99
IFNγ	94	100
IL-1α	94	94
IL-17F	91	103
IL-1β	93	102
IL-2	83	89
IFNβ	95	100
IL-4	89	92
IL-3	94	98
IL-5	83	95
IL-18	87	84
IL-6	89	97
FGF-2	96	96
IL-7	94	98
EGF	97	104
IL-9	93	96
IFNα	91	98
IL-28B/IFNλ3	89	105
IL-10	93	100
MIP-3α	95	103
IL-12p40	91	96
Granzyme B	93	109
IL-12p70	92	91

Analyte	Overnight Protocol % Recovery in Serum Matrix	2-Hour Protocol % Recovery in Serum Matrix
TARC	91	102
IL-16	95	101
LIF	90	101
IL-13	92	99
LIX	92	99
IL-15	88	102
Exodus-2/CCL21/6Ckine	100	110
IL-17A	89	92
IP-10	92	103
Fas	92	102
IL–20	99	94
KC	88	103
MCP-1	92	93
MIP-3β	93	101
MIP-1α	97	93
MDC	90	96
MIP-1β	97	94
M-CSF	93	98
sCD137/4-1BB/TNFRSF9	95	104
sICAM-1	89	90
CXCL16	94	106
MCP-5	91	103
MIP-2	87	92
MIG	93	99
RANTES	93	97
VEGF-A	94	108
TNFα	90	92
IL-11	90	94
sRAGE	92	99

Analyte	Overnight Protocol % Recovery in Serum Matrix	2-Hour Protocol % Recovery in Serum Matrix
CCL25/TECK	91	104
BAFF	91	102
GDF-15	95	99
FLT3L	94	99
CHI3L1	95	102
MCP-2	92	97
MCP-3	91	100
Erythropoietin	93	102
Fractalkine/CX3CL1	92	104
IL-31	92	100
Betacellulin	96	106

Troubleshooting

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 or to the recommended filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height to the kit solid plate or to the recommended filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height 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 PLUS in each well and 75 µL should be aspirated.
		When reading the assay on xMAP® INTELLIFLEX, adjust probe height based on the type of plate you are using, place an alignment disk or an alignment sphere in the well according to the protocol recommended by Luminex®.

Problem	Probable Cause	Solution
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 (for example, 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 (for example 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 PLUS 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 (for example, 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.

Problem	Probable Cause	Solution
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.

Product Ordering

Order products online at SigmaAldrich.com.

Replacement Reagents	Note	Catalogue No.
Mouse Cytokine Expansion Panel 1 Standard 1	For configurable kit	MCYT1-8190-1
Mouse Cytokine Expansion Panel 1 Standard 2	For 41-plex, 68-plex, or configurable kit	MCYT1-8190-2
Mouse Cytokine Expansion Panel 1 QC 1 & 2 for Standard 1	For configurable kit	MCYT1-6190-1
Mouse Cytokine Expansion Panel 1 QC 1 & 2 for Standard 2	For 41-plex, 68-plex, or configurable kit	MCYT1-6190-2
Serum Matrix	-	MXMSM-MCYT1
Mouse Cytokine Expansion Panel 1 Detection Antibodies 1	For configurable kit	MCYT1-1190-1
Mouse Cytokine Expansion Panel 1 Detection Antibodies 2	For 41-plex, 68-plex, or configurable kit	MCYT1-1190-2
Streptavidin-Phycoerythrin	-	L-SAPE20
Assay Buffer	-	L-AB
Set of two 96-Well plates with sealers	-	MAG-PLATE
10X Wash Buffer	-	L-WB
Mouse Cytokine Expansion Panel 1 S-Plex Premixed Beads*	-	MCYT1PMXS-MG
Mouse Cytokine Expansion Panel 1 L-Plex Premixed Beads*	-	MCYT1PMXL-MG
MILLIPLEX® Mouse Cytokine/ Chemokine/GF Exp Pnl 1 Sm-Plex PMX	-	MCYT1-190K-SPX
MILLIPLEX® Mouse Cytokine/ ChemokineGF Exp Pnl 1 L-Plex PMX	-	MCYT1-190K-LPX
MILLIPLEX® Mu Cytokine/Chemokine/ GF Exp Pnl 1 BK Sm-Plex PMX	BULK PACKAGING	MCYT1-190K-SPXBK
MILLIPLEX® Mu Cytokine/Chemokine/ GF Exp Pnl 1 BLK L-Plex PMX	BULK PACKAGING	MCYT1-190K-LPXBK

* For individual beads, see next page.

Antibody-Immobilized Magnetic Beads

Bead/Analyte Name	Bead No.	Catalogue No
Anti-Mouse sFasL Bead	9	MSFASL-MG
Anti-Mouse IL-22 Bead	12	MIL22-MG
Anti-Mouse G-CSF Bead	13	MGCSF-MG
Anti-Mouse Eotaxin/CCL11 Bead	14	MCCL11-MG
Anti-Mouse GM-CSF Bead	15	MGMCSF-MG
Anti-Mouse IL-33 Bead	18	MIL33-MG
Anti-Mouse IFNγ Bead	19	MIFNY-MG
Anti-Mouse IL-1α Bead	21	MIL1A-MG
Anti-Mouse IL-17F Bead	22	MIL17F-MG
Anti-Mouse IL-1β Bead	25	MIL1B-MG
Anti- Mouse IL-2 Bead	26	MIL2-MG
Anti-Mouse IFNβ Bead	27	MIFNB-MG
Anti-Mouse IL-4 Bead	28	MIL4-MG
Anti-Mouse IL-3 Bead	29	MIL3-MG
Anti-Mouse IL-5 Bead	30	MIL5-MG
Anti-Mouse IL-18 Bead	33	MIL18-MG
Anti-Mouse IL-6 Bead	34	MIL6-MG
Anti-Mouse FGF-2 Bead	35	MFGF2-MG
Anti-Mouse IL-7 Bead	36	MIL7-MG
Anti-Mouse EGF Bead	37	MEGF-MG
Anti-Mouse IL-9 Bead	38	MIL9-MG
Anti-Mouse IFNα Bead	39	MIFNA-MG
Anti-Mouse IL-28B/IFNλ3 Bead	42	MIL28B-MG
Anti-Mouse IL-10 Bead	43	MIL10-MG
Anti-Mouse MIP-3α Bead	44	MMIP3A-MG
Anti-Mouse IL-12p40 Bead	45	MIL12P40-MG
Anti-Mouse Granzyme B Bead	46	MGZMB-MG

Bead/Analyte Name	Bead No.	Catalogue No
Anti-Mouse IL-12p70 Bead	47	MIL12P70-MG
Anti-Mouse TARC Bead	48	MTARC-MG
Anti-Mouse IL-16 Bead	49	MIL16-MG
Anti-Mouse LIF Bead	51	MLIF-MG
Anti-Mouse IL-13 Bead	52	MIL13-MG
Anti-Mouse LIX Bead	53	MLIX-MG
Anti-Mouse IL-15 Bead	54	MIL15-MG
Anti-Mouse Exodus-2/ CCL21/6Ckine Bead	55	MCCL21-MG
Anti-Mouse IL-17A Bead	56	MIL17A-MG
Anti-Mouse IP-10 Bead	57	MIP10-MG
Anti-Mouse Fas Bead	58	MFAS-MG
Anti-Mouse IL–20 Bead	59	MIL20-MG
Anti-Mouse KC Bead	61	MKC-MG
Anti-Mouse MCP-1 Bead	62	MMCP1-MG
Anti-Mouse MIP-3β Bead	63	MMIP3B-MG
Anti-Mouse MIP-1α Bead	64	MMIP1A-MG
Anti-Mouse MDC Bead	65	MMDC-MG
Anti-Mouse MIP-1 beta Bead	66	MMIP1B-MG
Anti-Mouse M-CSF Bead	67	MMCSF-MG
Anti-Mouse sCD137/4-1BB/ TNFRSF9 Bead	68	MCD137-MG
Anti-Mouse sICAM-1 Bead	69	MSICAM1-MG
Anti-Mouse CXCL16 Bead	70	MCXCL16-MG
Anti-Mouse MCP-5 Bead	72	MMCP5-MG
Anti-Mouse MIP-2 Bead	73	MMIP2-MG
Anti-Mouse MIG Bead	74	MMIG-MG
Anti-Mouse RANTES Bead	75	MRANTES-MG
Anti-Mouse VEGF-A Bead	76	MVEGFA-MG

Bead/Analyte Name	Bead No.	Catalogue No
Anti-Mouse TNFα Bead	77	MTNFA-MG
Anti-Mouse IL-11 Bead	78	MIL11-MG
Anti-Mouse sRAGE Bead	79	MSRAGE-MG
Anti-Mouse CCL25/TECK Bead	80	MCCL25-MG
Anti-Mouse BAFF Bead	81	MBAFF-MG
Anti-Mouse GDF-15 Bead	82	MGDF15-MG
Anti-Mouse FLT3L Bead	83	MFLT3L-MG
Anti-Mouse CHI3L1 Bead	84	MCHI3L1-MG
Anti-Mouse MCP-2 Bead	85	MMCP2-MG
Anti-Mouse MCP-3 Bead	86	MMCP3-MG
Anti-Mouse Erythropoietin Bead	87	MEPO-MG
Anti-Mouse Fractalkine/ CX3CL1 Bead	88	MCX3CL1-MG
Anti-Mouse IL-31 Bead	89	MIL31-MG
Anti-Mouse Betacellulin Bead	90	MBTC-MG

Analyte/ Bead Name	Luminex® Magnetic Bead Region	Customizable 68 Analytes (70X concentration, 70 µL)		MCYT1PMXS-MG (41-Plex Premixed Beads)	MCYT1PMXL-MG (68-Plex Premixed Beads)	MCYT1-8190-1 (35-plex Standard Mix)	MCYT1-8190-2 (68-plex Standard Mix)	MCYT1-1190-1 (35-plex Detection Mix)	MCYT1-1190-2 (68-plex Detection Mix)
		Available	Catalog Number						
sFasL	9	✔	MSFASL-MG		✔		✔		✔
IL-22	12	✔	MIL22-MG	✔	✔		✔		✔
G-CSF	13	✔	MGCSF-MG	✔	✔	✔	✔	✔	✔
Eotaxin/CCL11	14	✔	MCCL11-MG	✔	✔	✔	✔	✔	✔
GM-CSF	15	✔	MGMCSF-MG	✔	✔	✔	✔	✔	✔
IL-33	18	✔	MIL33-MG	✔	✔		✔		✔
IFNγ	19	✔	MIFNY-MG	✔	✔	✔	✔	✔	✔
IL-1α	21	✔	MIL1A-MG	✔	✔	✔	✔	✔	✔
IL-17F	22	✔	MIL17F-MG	✔	✔		✔		✔
IL-1β	25	✔	MIL1B-MG	✔	✔	✔	✔	✔	✔
IL-2	26	✔	MIL2-MG	✔	✔	✔	✔	✔	✔
IFNβ	27	✔	MIFNB-MG	✔	✔	✔	✔	✔	✔
IL-4	28	✔	MIL4-MG	✔	✔	✔	✔	✔	✔
IL-3	29	✔	MIL3-MG	✔	✔	✔	✔	✔	✔
IL-5	30	✔	MIL5-MG	✔	✔	✔	✔	✔	✔
IL-18	33	✔	MIL18-MG	✔	✔	✔	✔	✔	✔
IL-6	34	✔	MIL6-MG	✔	✔	✔	✔	✔	✔
FGF-2	35	✔	MFGF2-MG	✔	✔		✔		✔

Analyte/ Bead Name	Luminex® Magnetic Bead Region	Customizable 68 Analytes (70X concentration, 70 µL)		MCYT1PMXS-MG (41-Plex Premixed Beads)	MCYT1PMXL-MG (68-Plex Premixed Beads)	MCYT1-8190-1 (35-plex Standard Mix)	MCYT1-8190-2 (68-plex Standard Mix)	MCYT1-1190-1 (35-plex Detection Mix)	MCYT1-1190-2 (68-plex Detection Mix)
		Available	Catalog Number						
IL-7	36	✔	MIL7-MG	✔	✔	✔	✔	✔	✔
EGF	37	✔	MEGF-MG	✔	✔		✔		✔
IL-9	38	✔	MIL9-MG	✔	✔	✔	✔	✔	✔
IFNα	39	✔	MIFNA-MG	✔	✔	✔	✔	✔	✔
IL-28B/IFNλ3	42	✔	MIL28B-MG	✔	✔		✔		✔
IL-10	43	✔	MIL10-MG	✔	✔	✔	✔	✔	✔
MIP-3α	44	✔	MMIP3A-MG		✔		✔		✔
IL-12p40	45	✔	MIL12P40-MG	✔	✔	✔	✔	✔	✔
Granzyme B	46	✔	MGZMB-MG		✔		✔		✔
IL-12p70	47	✔	MIL12P70-MG	✔	✔	✔	✔	✔	✔
TARC	48	✔	MTARC-MG		✔		✔		✔
IL-16	49	✔	MIL16-MG		✔		✔		✔
LIF	51	✔	MLIF-MG	✔	✔	✔	✔	✔	✔
IL-13	52	✔	MIL13-MG	✔	✔	✔	✔	✔	✔
LIX	53	✔	MLIX-MG	✔	✔	✔	✔	✔	✔
IL-15	54	✔	MIL15-MG	✔	✔	✔	✔	✔	✔
Exodus-2/ CCL21/6Ckine	55	✔	MCCL21-MG		✔		✔		✔
IL-17A	56	✔	MIL17A-MG	✔	✔	✔	✔	✔	✔

Analyte/ Bead Name	Luminex® Magnetic Bead Region	Customizable 68 Analytes (70X concentration, 70 µL)		MCYT1PMXS-MG (41-Plex Premixed Beads)	MCYT1PMXL-MG (68-Plex Premixed Beads)	MCYT1-8190-1 (35-plex Standard Mix)	MCYT1-8190-2 (68-plex Standard Mix)	MCYT1-1190-1 (35-plex Detection Mix)	MCYT1-1190-2 (68-plex Detection Mix)
		Available	Catalog Number						
IP-10	57	✔	MIP10-MG	✔	✔	✔	✔	✔	✔
Fas	58	✔	MFAS-MG		✔		✔		✔
IL–20	59	✔	MIL20-MG		✔		✔		✔
KC	61	✔	MKC-MG	✔	✔	✔	✔	✔	✔
MCP-1	62	✔	MMCP1-MG	✔	✔	✔	✔	✔	✔
MIP-3β	63	✔	MMIP3B-MG		✔		✔		✔
MIP-1α	64	✔	MMIP1A-MG	✔	✔	✔	✔	✔	✔
MDC	65	✔	MMDC-MG		✔		✔		✔
MIP-1β	66	✔	MMIP1B-MG	✔	✔	✔	✔	✔	✔
M-CSF	67	✔	MMCSF-MG	✔	✔	✔	✔	✔	✔
sCD137/4-1BB/ TNFRSF9	68	✔	MCD137-MG		✔		✔		✔
sICAM-1	69	✔	MSICAM1-MG		✔		✔		✔
CXCL16	70	✔	MCXCL16-MG		✔		✔		✔
MCP-5	72	✔	MMCP5-MG		✔		✔		✔
MIP-2	73	✔	MMIP2-MG	✔	✔	✔	✔	✔	✔
MIG	74	✔	MMIG-MG	✔	✔	✔	✔	✔	✔
RANTES	75	✔	MRANTES-MG	✔	✔	✔	✔	✔	✔
VEGF-A	76	✔	MVEGFA-MG	✔	✔	✔	✔	✔	✔

Analyte/ Bead Name	Luminex® Magnetic Bead Region	Customizable 68 Analytes (70X concentration, 70 µL)		MCYT1PMXS-MG (41-Plex Premixed Beads)	MCYT1PMXL-MG (68-Plex Premixed Beads)	MCYT1-8190-1 (35-plex Standard Mix)	MCYT1-8190-2 (68-plex Standard Mix)	MCYT1-1190-1 (35-plex Detection Mix)	MCYT1-1190-2 (68-plex Detection Mix)
		Available	Catalog Number						
TNFα	77	✔	MTNFA-MG	✔	✔	✔	✔	✔	✔
IL-11	78	✔	MIL11-MG		✔		✔		✔
sRAGE	79	✔	MSRAGE-MG		✔		✔		✔
CCL25/TECK	80	✔	MCCL25-MG		✔		✔		✔
BAFF	81	✔	MBAFF-MG		✔		✔		✔
GDF-15	82	✔	MGDF15-MG		✔		✔		✔
FLT3L	83	✔	MFLT3L-MG		✔		✔		✔
CHI3L1	84	✔	MCHI3L1-MG		✔		✔		✔
MCP-2	85	✔	MMCP2-MG		✔		✔		✔
MCP-3	86	✔	MMCP3-MG		✔		✔		✔
Erythropoietin	87	✔	MEPO-MG		✔		✔		✔
Fractalkine/ CX3CL1	88	✔	MCX3CL1-MG		✔		✔		✔
IL-31	89	✔	MIL31-MG		✔		✔		✔
Betacellulin	90	✔	MBTC-MG		✔		✔		✔

Well Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 Standard (Background)	Standard 4	QC-1 Control	Etc.								
B	0 Standard (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									

Notice

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Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

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Safety Data Sheets (SDS)

Safety Data Sheets are available on the product page at SigmaAldrich.com.

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