User Guide

MILLIPLEX® Humanized Mouse Magnetic Bead Panel

96-Well Plate Assay

HUMU-210K, HUMU-210K-PMX, HUMU-210K-PMXBK

Introduction

Humanized mouse research has emerged as a pivotal tool in biomedical science, bridging the gap between preclinical studies and human disease. By engrafting human tissues, cells, or genes into immunocompromised mice, researchers can create models that more accurately mimic human immune responses and disease mechanisms. This innovative approach enhances the relevance of immunoassays, which are critical for evaluating immune function, disease progression, and therapeutic efficacy. As the demand for more predictive models in drug development and disease research grows, humanized mice are proving invaluable for advancing our understanding of human biology and improving therapeutic strategies.

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® 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® Humanized Mouse Panel thus enables you to focus on simultaneous measurement of human and mouse cytokines and chemokines in humanized 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® Humanized Mouse Panel is part of the most versatile system available for the research of humanized mouse models. 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.

This MILLIPLEX® kit offers you:

- The ability to select a 41-plex panel.
- The ability to choose any combination of analytes from our panel of 41 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.

The MILLIPLEX® Humanized Mouse Panel is a 41-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: Human CXCL9/MIG, Mouse CXCL9/MIG, Human CXCL10/IP-10, Mouse CXCL10/IP-10, Human G-CSF, Mouse G-CSF, Human GM-CSF, Mouse GM-CSF, Human IL-1α, Human IL-1β, Mouse IL-1β, Human IFNγ, Mouse IFN-γ, Human IL-2, Mouse IL-2, Human IL-3, Mouse IL-3, Human IL-4, Human IL-5, Mouse IL-5, Human IL-6, Mouse IL-6, Human IL-7, Human IL-8, Mouse KC, Human IL-10, Human IL-12p40, Mouse IL-12p40, Human IL-12p70, Mouse IL-12p70, Human IL-15, Human IL-17A, Mouse IL-17A, Human IL-18, Mouse IL-18, Human MCP-1, Mouse MCP-1, Human M-CSF, Mouse M-CSF, Human TNFα, and Mouse TNFα.

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 the 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 Number
Humanized Mouse Panel Standard	1 vial	Lyophilized	HUMU-8210
Humanized Mouse Panel Quality Controls 1 and 2	1 vial each	Lyophilized	HUMU-6210
Serum Matrix	1 vial	Lyophilized	MXSM-HUMU
Set of one 96-Well Plate with 2 sealers	1 plate 2 sealers	-	-
Assay Buffer	1 bottle	30 mL	LAB-7
10X Wash Buffer	1 bottle	60 mL	L-WB
Humanized Mouse Panel Detection Antibodies	1 bottle	5.5 mL	HUMU-1210
Bead Diluent *(not provided with premixed panel)*	1 bottle	3.5 mL	HUMU-BD
Streptavidin-Phycoerythrin	1 bottle	5.5 mL	L-SAPE21
Mixing Bottle *(not provided with premixed panel)*	1 bottle	-	-

Humanized Mouse Antibody-Immobilized Premixed Magnetic Beads

Included Humanized Mouse Panel Antibody-Immobilized Beads are dependent on customizable selection of analytes within the panel (see below).

Premixed 41-plex Beads	3.5 mL	1 bottle	HUMUPMX-MAG

Humanized Mouse Panel Antibody-Immobilized Magnetic Beads

Bead/Analyte Name	Luminex® Magnetic Bead Region	Configurable 41 Analytes (50X concentration, 90 µL) Available	41-Plex Magnetic Premixed Beads	Catalogue Number
Anti-Human G-CSF Bead	12	✓	✓	HUGCSF-MAG
Anti-Mouse G-CSF Bead	13	✓	✓	MUGCSF-MAG
Anti-Human GM-CSF Bead	14	✓	✓	HUGMCSF-MAG
Anti-Mouse GM-CSF Bead	15	✓	✓	MUGMCSF-MAG
Anti-Human IFN-γ Bead	18	✓	✓	HUIFNY-MAG
Anti-Mouse IFN-γ Bead	19	✓	✓	MUIFNY-MAG
Anti-Human IL-1α Bead	20	✓	✓	HUIL1A-MAG
Anti-Human IL-1β Bead	21	✓	✓	HUIL1B-MAG
Anti-Mouse IL-1β Bead	22	✓	✓	MUIL1B-MAG
Anti-Human IL-2 Bead	25	✓	✓	HUIL2-MAG
Anti-Mouse IL-2 Bead	26	✓	✓	MUIL2-MAG
Anti-Human IL-3 Bead	27	✓	✓	HUIL3-MAG
Anti-Mouse IL-3 Bead	29	✓	✓	MUIL3-MAG
Anti-Human IL-4 Bead	30	✓	✓	HUIL4-MAG
Anti-Human IL-5 Bead	33	✓	✓	HUIL5-MAG
Anti-Mouse IL-5 Bead	34	✓	✓	MUIL5-MAG
Anti-Human IL-6 Bead	35	✓	✓	HUIL6-MAG
Anti-Mouse IL-6 Bead	36	✓	✓	MUIL6-MAG
Anti-Human IL-7 Bead	37	✓	✓	HUIL7-MAG
Anti-Human IL-8 Bead	38	✓	✓	HUIL8-MAG
Anti-Mouse KC Bead	39	✓	✓	MUKC-MAG
Anti-Human IL-12p40 Bead	43	✓	✓	HUIL12P40-MAG

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Bead/Analyte Name	Luminex® Magnetic Bead Region	Configurable 41 Analytes (50X concentration, 90 µL) Available	41-Plex Magnetic Premixed Beads	Catalogue Number
Anti-Mouse IL-12p40 Bead	44	✓	✓	MUIL12P40-MAG
Anti-Human IL-12p70 Bead	45	✓	✓	HUIL12P70-MAG
Anti-Mouse IL-12p70 Bead	46	✓	✓	MUIL12P70-MAG
Anti-Human IL-15 Bead	47	✓	✓	HUIL15-MAG
Anti-Human IL-17A Bead	48	✓	✓	HUIL17A-MAG
Anti-Mouse IL-17A Bead	51	✓	✓	MUIL17A-MAG
Anti-Human IL-18 Bead	54	✓	✓	HUIL18-MAG
Anti-Mouse IL-18 Bead	55	✓	✓	MUIL18-MAG
Anti-Human IL-10 Bead	56	✓	✓	HUIL10-MAG
Anti-Human M-CSF Bead	61	✓	✓	HUMCSF-MAG
Anti-Mouse M-CSF Bead	62	✓	✓	MUMCSF-MAG
Anti-Human MCP-1 Bead	63	✓	✓	HUMCP1-MAG
Anti-Mouse MCP-1 Bead	64	✓	✓	MUMCP1-MAG
Anti-Human MIG Bead	65	✓	✓	HUMIG-MAG
Anti-Mouse MIG Bead	66	✓	✓	MUMIG-MAG
Anti-Human IP-10 Bead	72	✓	✓	HUIP10-MAG
Anti-Mouse IP-10 Bead	73	✓	✓	MUIP10-MAG
Anti-Human TNFα Bead	76	✓	✓	HUTNFA-MAG
Anti-Mouse TNFα Bead	77	✓	✓	MUTNFA-MAG

Materials Required (Not provided)

Reagents

MAGPIX® Drive Fluid PLUS (40-50030), xMAP® Sheath Fluid PLUS (40-50021), or xMAP® Sheath Concentrate PLUS (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, 12620-926 or equivalent)
- Luminex® 200™, HTS, 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, Cat. Nos. 40-094, 40-095, 40-096, 40-097 or equivalent) or Handheld Magnetic Separation Block (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 (MX-PLATE) to run the assay using a Vacuum Filtration Unit (Vacuum Manifold MSVMHTS00 or equivalent with Vacuum Pump 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.

Symbol Definitions

Ingredient	Catalogue Number	Label	
Humanized Mouse Panel Standard	HUMU-8210		**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 Number	Label	
Humanized Mouse Panel Quality Control 1 & 2	HUMU-6210		**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	MXSM-HUMU	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 Number	Label	
Humanized Mouse Panel Detection Antibodies	HUMU-1210		**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-SAPE21		**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.
Bead Diluent	HUMU-BD		**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.
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 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.

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 neat. When further dilution 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 neat. When further dilution 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 10 µL per well of neat 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 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 10 antibody-immobilized beads, add 60 µL from each of the 10 bead vials to the Mixing Bottle. Then add 2.4 mL Bead Diluent.
>
> Example 2: When using 35 antibody-immobilized beads, add 60 µL from each of the 35 bead vials to the Mixing Bottle. Then add 0.9 mL Bead Diluent.

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 and set in an ice bath. These should be added to the plate within 1 hour of reconstitution. 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 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.

Preparation of Humanized Mouse Standard

Prior to use, reconstitute the Humanized Mouse 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. This reconstituted standard and the serially diluted standards in the following steps should be set in an ice bath during the assay procedure. These need to be added to the plate within 1 hour of preparation. The unused portion may be stored at ≤ –20 °C for up to one month.

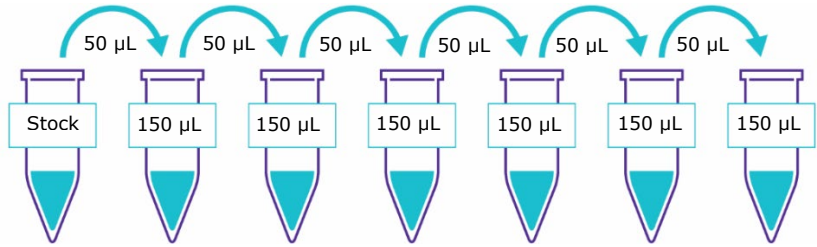
Preparation of Working Standards

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 50µL 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



Reconstituted Standard 7 | Standard 6 | Standard 5 | Standard 4 | Standard 3 | Standard 2 | Standard 1

Standard	hIFNɣ (pg/mL)	mTNFα (pg/mL)	mIL-1β (pg/mL)
Standard 1	0.6	0.7	0.9
Standard 2	2.4	2.9	3.9
Standard 3	10	12	16
Standard 4	39	47	63
Standard 5	156	188	250
Standard 6	625	750	1,000
Standard 7	2,500	3,000	4,000

Standard	mGM-CSF, hIL-8, hIL-18, hIL-1β, hIL-2, hIL-6, hIL-17A (pg/mL)	mM-CSF (pg/mL)	hTNFα (pg/mL)
Standard 1	1.2	1.7	1.9
Standard 2	4.9	6.8	7.8
Standard 3	20	27	31
Standard 4	78	109	125
Standard 5	313	438	500
Standard 6	1,250	1,750	2,000
Standard 7	5,000	7,000	8,000

Standard	mIL-2, mIL-3, hIL-4, hIL-5, mIL-12p70, hIL-15, mIP-10 (pg/mL)	mIFNγ, hIL-7 (pg/mL)	hIL-1α, mIL-5, mIL-17A, hIL-10 (pg/mL)
Standard 1	2.4	2.9	4.9
Standard 2	9.8	12	20
Standard 3	39	47	78
Standard 4	156	188	313
Standard 5	625	750	1,250
Standard 6	2,500	3,000	5,000
Standard 7	10,000	12,000	20,000

Standard	mMCP-1 (pg/mL)	mKC, mIL-12p40, mIL-18, mMIG, hIP-10 (pg/mL)	hGM-CSF (pg/mL)
Standard 1	6.1	7.3	9.8
Standard 2	24	29	39
Standard 3	98	117	156
Standard 4	391	469	625
Standard 5	1,563	1,875	2,500
Standard 6	6,250	7,500	10,000
Standard 7	25,000	30,000	40,000

Standard	mIL-6, hIL-12p70 (pg/mL)	hMCP-1 (pg/mL)	hIL-3, hIL-12p40 (pg/mL)
Standard 1	15	18	20
Standard 2	59	73	78
Standard 3	234	293	313
Standard 4	938	1,172	1,250
Standard 5	3,750	4,688	5,000
Standard 6	15,000	18,750	20,000
Standard 7	60,000	75,000	80,000

Standard	hMIG (pg/mL)	hG-CSF (pg/mL)	hM-CSF (pg/mL)
Standard 1	29	37	49
Standard 2	117	146	195
Standard 3	469	586	781
Standard 4	1,875	2,344	3,125
Standard 5	7,500	9,375	12,500
Standard 6	30,000	37,500	50,000
Standard 7	120,000	150,000	200,000

Standard	mG-CSF (pg/mL)
Standard 1	98
Standard 2	391
Standard 3	1,563
Standard 4	6,250
Standard 5	25,000
Standard 6	100,000
Standard 7	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. 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).

1. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
2. Add 10 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for 0 standard (Background).
3. Add 10 µL of Assay Buffer to the sample wells.
4. Add 10 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.
5. Add 10 µL of Sample (neat) into the appropriate wells.
6. 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.
7. 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.

Add 200 µL Wash Buffer per well

Shake 10 min, RT

Decant

- Add 10 µL Standard or Control to appropriate wells
- Add 10 µL Assay Buffer to background and sample wells
- Add 10 µL appropriate matrix solution to background, standards, and control wells
- Add 10 µL neat Samples to sample wells
- Add 25 µL Beads to each well

Incubate overnight (16-18 hours) at 2-8 °C

8. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
9. Add 50 µL of Detection Antibodies into each well.

   Note: Allow the Detection Antibodies to warm to room temperature prior to addition.
10. 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.**
11. Add 50 µL Streptavidin-Phycoerythrin to each well containing the 50 µL of Detection Antibodies.
12. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25 °C).
13. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
14. 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.
15. Run plate on Luminex® 200™, FLEXMAP 3D®, MAGPIX® with xPONENT® software or xMAP® INTELLIFLEX with INTELLIFLEX Software.
16. 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.

Plate Washing

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

Solid Plate

- Handheld magnet (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 (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 (LX2R-CAL-K25)	Performance Verification Kit (LX2R-PVER-K25)
FLEXMAP 3D®	FLEXMAP 3D® Calibrator Kit (F3D-CAL-K25)	FLEXMAP 3D® Performance Verification Kit (F3D-PVER-K25)
xMAP® INTELLIFLEX	xMAP® INTELLIFLEX Calibration Kit (IFX-CAL-K20)	xMAP® INTELLIFLEX Performance Verification Kit (IFX-PVER-K20)
MAGPIX®	MAGPIX® Calibration Kit (MPX-CAL-K25)	MAGPIX® Performance Verification Kit (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 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	Configurable 41-plex Beads	
	Human G-CSF	12
	Mouse G-CSF	13
	Human GM-CSF	14
	Mouse GM-CSF	15
	Human IFN-γ	18
	Mouse IFN-γ	19
	Human IL-1α	20
	Human IL-1β	21

Bead Set	Configurable 41-plex Beads	
	Mouse IL-1β	22
	Human IL-2	25
	Mouse IL-2	26
	Human IL-3	27
	Mouse IL-3	29
	Human IL-4	30
	Human IL-5	33
	Mouse IL-5	34
	Human IL-6	35
	Mouse IL-6	36
	Human IL-7	37
	Human IL-8	38
	Mouse KC	39
	Human IL-12p40	43
	Mouse IL-12p40	44
	Human IL-12p70	45
	Mouse IL-12p70	46
	Human IL-15	47
	Human IL-17A	48
	Mouse IL-17A	51
	Human IL-18	54
	Mouse IL-18	55
	Human IL-10	56
	Human M-CSF	61
	Mouse M-CSF	62
	Human MCP-1	63
	Mouse MCP-1	64
	Human MIG	65
	Mouse MIG	66

Bead Set	Configurable 41-plex Beads	
	Human IP-10	72
	Mouse IP-10	73
	Human TNFα	76
	Mouse TNFα	77

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 with the exception of Human IL-5 on Human IL-12p40 beads at 11%.

Assay Sensitivities

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.

	Overnight Protocol (n = 11 Assays)	
Analyte	**MinDC (pg/mL)**	**MinDC+2SD (pg/mL)**
Human G-CSF	42.16	77.88
Mouse G-CSF	161.13	395.19
Human GM-CSF	1.83	3.76
Mouse GM-CSF	0.45	0.81
Human IFN-γ	0.99	1.71
Mouse IFN-γ	1.05	1.84
Human IL-1α	1.02	2.27
Human IL-1β	7.33	11.67

Analyte	Overnight Protocol (n = 11 Assays) MinDC (pg/mL)	MinDC+2SD (pg/mL)
Mouse IL-1β	0.44	0.84
Human IL-2	0.87	1.65
Mouse IL-2	0.66	1.32
Human IL-3	1.81	2.72
Mouse IL-3	1.63	2.75
Human IL-4	2.74	4.79
Human IL-5	0.39	0.65
Mouse IL-5	2.76	5.48
Human IL-6	4.62	7.63
Mouse IL-6	3.77	7.19
Human IL-7	1.08	1.92
Human IL-8	0.60	1.17
Mouse KC	0.43	0.77
Human IL-12p40	4.25	8.65
Mouse IL-12p40	0.80	1.79
Human IL-12p70	5.86	11.99
Mouse IL-12p70	1.69	2.93
Human IL-15	0.48	1.03
Human IL-17A	2.71	5.04
Mouse IL-17A	2.65	4.87
Human IL-18	0.74	1.11
Mouse IL-18	5.14	10.63
Human IL-10	9.27	20.47
Human M-CSF	1.94	3.38
Mouse M-CSF	3.12	6.63

Analyte	Overnight Protocol (n = 11 Assays) MinDC (pg/mL)	MinDC+2SD (pg/mL)
Human MCP-1	1.84	2.64
Mouse MCP-1	16.22	25.80
Human MIG	4.20	8.29
Mouse MIG	1.83	3.53
Human IP-10	59.24	84.84
Mouse IP-10	9.97	19.59
Human TNFα	1.05	1.89
Mouse TNFα	0.25	0.60

Precision

Intra-assay precision is generated from the mean of the %CV's 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.

Analyte	Overnight Protocol Intra-assay %CV	Inter-assay %CV
Human G-CSF	<10%	<15%
Mouse G-CSF	<10%	<15%
Human GM-CSF	<10%	<15%
Mouse GM-CSF	<10%	<15%
Human IFN-γ	<10%	<20%
Mouse IFN-γ	<10%	<15%
Human IL-1α	<10%	<15%
Human IL-1β	<10%	<15%
Mouse IL-1β	<10%	<15%

	Overnight Protocol	
Analyte	**Intra-assay %CV**	**Inter-assay %CV**
Human IL-2	<10%	<15%
Mouse IL-2	<10%	<15%
Human IL-3	<10%	<15%
Mouse IL-3	<10%	<15%
Human IL-4	<10%	<15%
Human IL-5	<10%	<15%
Mouse IL-5	<10%	<15%
Human IL-6	<10%	<15%
Mouse IL-6	<10%	<15%
Human IL-7	<10%	<15%
Human IL-8	<10%	<15%
Mouse KC	<10%	<15%
Human IL-12p40	<10%	<15%
Mouse IL-12p40	<10%	<15%
Human IL-12p70	<10%	<15%
Mouse IL-12p70	<10%	<15%
Human IL-15	<10%	<15%
Human IL-17A	<10%	<15%
Mouse IL-17A	<10%	<15%
Human IL-18	<10%	<15%
Mouse IL-18	<10%	<15%
Human IL-10	<10%	<15%
Human M-CSF	<10%	<15%
Mouse M-CSF	<10%	<15%
Human MCP-1	<10%	<15%

Analyte	Overnight Protocol Intra-assay %CV	Inter-assay %CV
Mouse MCP-1	<10%	<15%
Human MIG	<10%	<15%
Mouse MIG	<10%	<15%
Human IP-10	<10%	<15%
Mouse IP-10	<10%	<15%
Human TNFα	<10%	<15%
Mouse TNFα	<10%	<15%

Accuracy

Spike Recovery: The data represent mean percent recovery of spiked standards ranging from low, medium, and high concentration in serum matrices (n=8).

Analyte	Overnight Protocol % Recovery in Serum Matrix
Human G-CSF	91%
Mouse G-CSF	94%
Human GM-CSF	93%
Mouse GM-CSF	96%
Human IFN-γ	93%
Mouse IFN-γ	92%
Human IL-1α	95%
Human IL-1β	97%
Mouse IL-1β	88%
Human IL-2	93%
Mouse IL-2	99%
Human IL-3	88%
Mouse IL-3	93%
Human IL-4	94%

Analyte	Overnight Protocol % Recovery in Serum Matrix
Human IL-5	93%
Mouse IL-5	92%
Human IL-6	98%
Mouse IL-6	92%
Human IL-7	91%
Human IL-8	99%
Mouse KC	94%
Human IL-12p40	87%
Mouse IL-12p40	92%
Human IL-12p70	91%
Mouse IL-12p70	90%
Human IL-15	90%
Human IL-17A	93%
Mouse IL-17A	90%
Human IL-18	95%
Mouse IL-18	87%
Human IL-10	95%
Human M-CSF	96%
Mouse M-CSF	93%
Human MCP-1	96%
Mouse MCP-1	93%
Human MIG	92%
Mouse MIG	92%
Human IP-10	96%
Mouse IP-10	94%
Human TNFα	93%
Mouse TNFα	91%

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.
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.

Problem	Probable Cause	Solution
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.

Problem	Probable Cause	Solution
For Filter Plates Only		
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2 mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay set-up and use supernatant.
	High lipid concentration	After centrifugation, remove lipid layer and use supernatant.
Plate leaked	Vacuum pressure too high	Adjust vacuum pressure such that 0.2 mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (blocked) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate holder or raised edge so bottom of filter is not touching any surface.
	Insufficient blotting of filter plate bottom causing wicking	Blot the bottom of the filter plate well with absorbent towels after each wash step.
	Pipette touching plate filter during additions	Pipette to the side of plate.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
	Sample too viscous	May need to dilute sample.

Product Ordering

Order products online at SigmaAldrich.com.

Replacement Reagents	Catalogue Number
Humanized Mouse Panel Standard	HUMU-8210
Humanized Mouse Panel QC 1 & 2	HUMU-6210
Serum Matrix	MXSM-HUMU
Humanized Mouse Panel Det Abs	HUMU-1210
Bead Diluent	HUMU-BD
Streptavidin-Phycoerythrin	L-SAPE21
Assay Buffer	LAB-7
Set of two 96-Well plates with sealers	MAG-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Magnetic Beads

Bead/Analyte Name	Bead Number	Catalogue Number
Anti-Human G-CSF Bead	12	HUGCSF-MAG
Anti-Mouse G-CSF Bead	13	MUGCSF-MAG
Anti-Human GM-CSF Bead	14	HUGMCSF-MAG
Anti-Mouse GM-CSF Bead	15	MUGMCSF-MAG
Anti-Human IFN-γ Bead	18	HUIFNY-MAG
Anti-Mouse IFN-γ Bead	19	MUIFNY-MAG
Anti-Human IL-1α Bead	20	HUIL1A-MAG
Anti-Human IL-1β Bead	21	HUIL1B-MAG
Anti-Mouse IL-1β Bead	22	MUIL1B-MAG
Anti-Human IL-2 Bead	25	HUIL2-MAG
Anti-Mouse IL-2 Bead	26	MUIL2-MAG
Anti-Human IL-3 Bead	27	HUIL3-MAG
Anti-Mouse IL-3 Bead	29	MUIL3-MAG

Bead/Analyte Name	Bead Number	Catalogue Number
Anti-Human IL-4 Bead	30	HUIL4-MAG
Anti-Human IL-5 Bead	33	HUIL5-MAG
Anti-Mouse IL-5 Bead	34	MUIL5-MAG
Anti-Human IL-6 Bead	35	HUIL6-MAG
Anti-Mouse IL-6 Bead	36	MUIL6-MAG
Anti-Human IL-7 Bead	37	HUIL7-MAG
Anti-Human IL-8 Bead	38	HUIL8-MAG
Anti-Mouse KC Bead	39	MUKC-MAG
Anti-Human IL-12p40 Bead	43	HUIL12P40-MAG
Anti-Mouse IL-12p40 Bead	44	MUIL12P40-MAG
Anti-Human IL-12p70 Bead	45	HUIL12P70-MAG
Anti-Mouse IL-12p70 Bead	46	MUIL12P70-MAG
Anti-Human IL-15 Bead	47	HUIL15-MAG
Anti-Human IL-17A Bead	48	HUIL17A-MAG
Anti-Mouse IL-17A Bead	51	MUIL17A-MAG
Anti-Human IL-18 Bead	54	HUIL18-MAG
Anti-Mouse IL-18 Bead	55	MUIL18-MAG
Anti-Human IL-10 Bead	56	HUIL10-MAG
Anti-Human M-CSF Bead	61	HUMCSF-MAG
Anti-Mouse M-CSF Bead	62	MUMCSF-MAG
Anti-Human MCP-1 Bead	63	HUMCP1-MAG
Anti-Mouse MCP-1 Bead	64	MUMCP1-MAG
Anti-Human MIG Bead	65	HUMIG-MAG
Anti-Mouse MIG Bead	66	MUMIG-MAG

Bead/Analyte Name	Bead Number	Catalogue Number
Anti-Human IP10 Bead	72	HUIP10-MAG
Anti-Mouse IP10 Bead	73	MUIP10-MAG
Anti-Human TNFα Bead	76	HUTNFA-MAG
Anti-Mouse TNFα Bead	77	MUTNFA-MAG

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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Safety Data Sheets (SDS)

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