User Guide

MILLIPLEX® Mouse Soluble Cytokine Receptor Magnetic Bead Panel

96-Well Plate Assay

MSCRMAG-42K

Introduction

Cytokine receptors constitute an integral part of the cytokine biology. Like cytokines, cytokine receptors are involved in normal physiological and pathological processes of almost all disease states. Soluble cytokine receptors naturally arise from genes encoding membrane-bound receptors or are direct derivatives of the receptors themselves. The discovery that soluble cytokine receptors are involved in regulating excessive inflammatory responses and modulating immune events has stimulated significant research interest in their potential role as immunotherapeutic agents. Many of these soluble cytokine receptors have the ability to inhibit the binding and biological activity of their cytokine ligands, making them very specific cytokine antagonists.

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 panel and kit meets stringent manufacturing criteria to ensure batch-to-batch reproducibility. The MILLIPLEX® Mouse Soluble Cytokine Receptor Magnetic Bead Panel thus enables you to focus on the therapeutic potential of soluble cytokine receptors as well as the modulation of cytokine expression. Coupled with the Luminex® xMAP® platform in a magnetic bead format, you receive the advantage of ideal speed and sensitivity, allowing quantitative multiplex detection of dozens of analytes simultaneously, which can dramatically improve productivity.

The MILLIPLEX® Mouse Soluble Cytokine Receptor Magnetic Bead Panel is part of the most versatile system available for cytokine receptor 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® offers you:

- The ability to choose any combination of analytes from our panel of 13 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® Mouse Soluble Cytokine Receptor Panel is a 13-plex kit to be used for the simultaneous quantification in mouse serum, plasma, or tissue culture samples of any or all of the following analytes: sCD30, sgp130, sIL-1RI, sIL-1RII, sIL-2Rα, sIL-4R, sIL-6R, sRAGE, sTNFRI, sTNFRII, sVEGFR1, sVEGFR2, and sVEGFR3.

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® products 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®-C microspheres.

- Luminex® products use 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®), 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 new MILLIPLEX® Analyst 5.1, 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 Antibodies, and Streptavidin-Phycoerythrin.

Reagents Supplied

Store all reagents at 2–8 °C

Reagents	Volume	Quantity	Cat. No.
Mouse Soluble Cytokine Receptor Standard	Lyophilized	1 vial	MSCR8042
Mouse Soluble Cytokine Receptor Quality Controls 1 and 2	Lyophilized	2 vials	MSCR6042
Mouse Soluble Cytokine Receptor Detection Antibodies	3.2 mL	1 bottle	MSCR1042
Streptavidin-Phycoerythrin	3.2 mL	1 bottle	L-SAPE3
Serum Matrix*	Lyophilized	1 vial	LMC-SD
Assay Buffer*	30 mL	1 bottle	L-AB
10X Wash Buffer**	60 mL	1 bottle	L-WB
Set of one 96-Well Microtiter Plate with 2 Sealers	-	1 set	-
Mixing Bottle	-	1 bottle	-

* Contains 0.08% Sodium azide
** Contains 0.05% Proclin

Included Mouse Soluble Cytokine Receptor Antibody-Immobilized Beads are dependent on customizable selection of analytes within the panel.

Mouse Soluble Cytokine Receptor Antibody-Immobilized Beads

Bead/Analyte Name	Luminex® Bead Region	Customizable 13 Analytes (20X concentration, 200 µL) Available	Cat. No.
Anti-sCD30 Bead	13	✔	MSCD30-MAG
Anti-sgp130	15	✔	MSGP130-MAG
Anti-sIL-1RI	25	✔	MSIL1R1-MAG
Anti-sIL-1RII	28	✔	MSIL1R2-MAG
Anti-sIL-2Rα	39	✔	MSIL2RA-MAG
Anti-sIL-4R	43	✔	MSIL4R-MAG
Anti-sIL-6R	45	✔	MSIL6R-MAG
Anti-sRAGE	47	✔	MSRAGE-MAG
Anti-sTNFRI	62	✔	MSTNFR1-MAG
Anti-sTNFRII	66	✔	MSTNFR2-MAG
Anti-sVEGFR1	72	✔	MSVEGFR1-MAG
Anti-sVEGFR2	75	✔	MSVEGFR2-MAG
Anti-sVEGFR3	77	✔	MSVEGFR3-MAG

Materials Required (not included)

Reagents

MAGPIX® Drive Fluid PLUS (Cat. No. 40-50030), xMAP® Sheath Fluid PLUS (Cat. No. 40-50021), or xMAP® Sheath Concentrate PLUS (Cat. 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 B200 or equivalent)
- Titer plate shaker (VWR® Microplate Shaker Cat. No. 12620-926 or equivalent)
- Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® instrument with xPONENT® software, or xMAP® INTELLIFLEX instrument with INTELLIFLEX software by Luminex® Corporation
- Automatic plate washer for magnetic beads (BioTek® 405 LS and 405 TS, Cat. No. 40-094, 40-095, 40-096, 40-097 or equivalent) or Handheld Magnetic Separation Block (Cat. No. 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 (Cat. No. MX-PLATE) to run the assay using a vacuum filtration unit (Vacuum Manifold, Cat. No. MSVMHTS00 or equivalent with Vacuum Pump, Cat. No. 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	Cat. No.	Label	
Mouse Soluble Cytokine Receptor Detection Antibodies	MSCR1042		**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.
Mouse Soluble Cytokine Receptor Quality Controls 1 & 2	MSCR6042	 	**Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye 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.
Mouse Soluble Cytokine Receptor Standard	MSCR8042	 	**Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye 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.
Serum Matrix	LMC-SD	No Symbol Required	Harmful to aquatic life with long lasting effects. Avoid release to the environment.
Streptavidin-Phycoerythrin	L-SAPE3		**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.
- Does 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 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 the Luminex® 200™ instrument, 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 the MAGPIX® instrument, 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 the FLEXMAP 3D® instrument, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate using 1 alignment disc.
- For the FLEXMAP 3D® instrument, 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, 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.
- Customers need to determine the optimal dilution factor for their samples. Generally, serum samples from normal subjects should be diluted 1:5 using the Assay Buffer provided in the kit as the sample diluent (20 µL sample mixed with 80 µL Assay Buffer). If samples require dilution beyond 1:5, use the Assay Buffer provided in the kit, and the matrix in the curve should be diluted equivalently.

Preparation of Plasma Samples

- Plasma collection using EDTA as an anticoagulant is recommended. Centrifuge for 10 minutes at 1000 x g within 30 minutes of blood collection. Remove plasma from the tube 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.
- Customers need to determine the optimal dilution factor for their samples. Generally, plasma samples from normal subjects should be diluted 1:5 using the Assay Buffer provided in the kit as the sample diluent (20 µL sample mixed with 80 µL Assay Buffer). If samples require dilution beyond 1:5, use the Assay Buffer provided in the kit, and the matrix in the curve should be diluted equivalently.

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 samples may require a dilution with an appropriate control medium prior to assay. Users need to provide the control medium as the sample diluent.

NOTE:

- A maximum of 25 µL per well of tissue extract, cell/tissue culture supernatant sample, or 1:5 diluted serum or plasma sample can be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anticoagulant 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

Sonicate each antibody-bead vial for 30 seconds then vortex for 1 minute. Add 150 µL from each antibody-bead vial to the Mixing Bottle and bring final volume to 3.0 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 3 antibody-immobilized beads, add 150 µL from each of the 3 bead sets to the Mixing Bottle. Then add 2.55 mL Assay Buffer.

Example 2: When using 13 antibody-immobilized beads, add 150 µL from each of the 13 bead sets to the Mixing Bottle. Then add 1.05 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 then vortex briefly. 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 portions 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 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 1.0 mL deionized water to the bottle containing lyophilized Serum Matrix. Then add 4.0 mL Assay Buffer. Mix well. Allow at least 10 minutes for complete reconstitution.

Preparation of Mouse Soluble Cytokine Receptor Standard

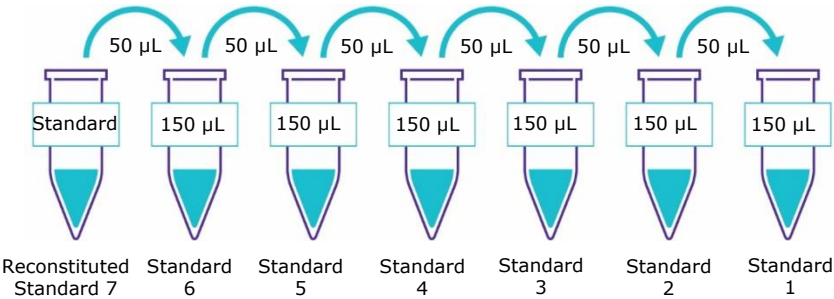
1. Reconstitute the Mouse Soluble Cytokine Receptor Panel Standard with 250 µL deionized water. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes and then transfer the standard to a polypropylene microfuge tube labeled "Standard 7." The unused portion may be stored at ≤ -20 °C for up to one month.

2. Preparation of Working Standards
   Label six polypropylene microfuge tubes Standard 6, Standard 5, Standard 4, Standard 3, Standard 2 and Standard 1. Add 150 µL of Assay Buffer to each of the six tubes. Prepare 1:4 serial dilutions by adding 50 µL of the Reconstituted Standard 7 to the Standard 6 tube, mix well and transfer 50 µL of the Standard 6 tube to the Standard 5 tube, mix well and transfer 50 µL of the Standard 5 tube to the Standard 4 tube, mix well and transfer 50 µL of the Standard 4 tube to the Standard 3 tube, mix well and transfer 50 µL of the Standard 3 tube to the Standard 2 tube, mix well and transfer 50 µL of the Standard 2 tube to the Standard 1 tube and mix well. The 0 pg/mL Standard (Background) will be Assay Buffer.

Standard	Add Deionized Water (µL)	Add Standard (volume)
Standard 7 (Reconstituted Standard)	250	0

Standard	Add Assay Buffer (µL)	Add Standard (volume)
Standard 6	150	50 µL of Standard 7
Standard 5	150	50 µL of Standard 6
Standard 4	150	50 µL of Standard 5
Standard 3	150	50 µL of Standard 4
Standard 2	150	50 µL of Standard 3
Standard 1	150	50 µL of Standard 2

Preparation of Standards



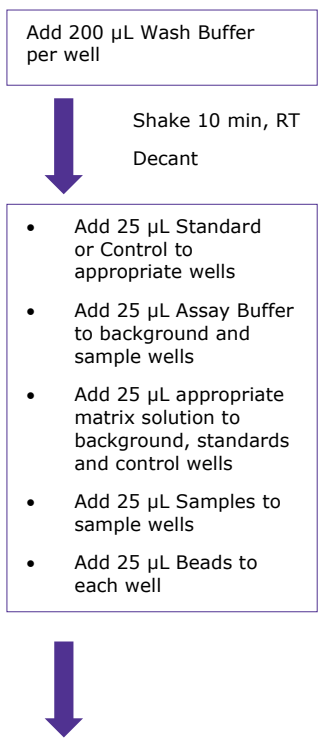
After dilution, each tube has the following concentrations for each analyte:

Standard Tube No.	sTNFRI (pg/mL)	sIL-1RI, sIL-1RII, sIL-2Rα, sIL-4R, sTNFRII (pg/mL)	sCD30, sgp130, sIL-6R, sRAGE, sVEGFR1, sVEGFR2, sVEGFR3 (pg/mL)
Standard 1	4.9	12.2	24.4
Standard 2	19.5	48.8	97.7
Standard 3	78.1	195.3	390.6
Standard 4	312.5	781.3	1,562.5
Standard 5	1,250	3,125	6,250
Standard 6	5,000	12,500	25,000
Standard 7	20,000	50,000	100,000

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 Background, Standards [0 (Background), Standard 1, Standard 2, Standard 3, Standard 4, Standard 5, Standard 6 and Standard 7], Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration.
  (**Note:** Most instruments will only read the 96-well plate vertically by default.)
  It is recommended to run the samples in duplicate.
- If using a filter plate, set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

1. Add 200 µL of Wash Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25 °C).
2. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for 0 pg/mL Standard (Background).
4. Add 25 µL of Assay Buffer to the sample wells.
5. Add 25 µL of appropriate matrix solution to the background, standards, and control wells. When assaying diluted serum or plasma samples use the Serum Matrix provided in the kit. When assaying tissue/cell extract or tissue/cell culture medium samples use identical extraction buffer or control medium as the matrix solution.
6. Add 25 µL of Sample into the appropriate wells [samples may require dilution (1:5 or greater for serum and plasma) – refer to sample collection and storage section for diluting samples prior to addition to plate].
7. Vortex Mixing Bottle and add 25 µL of the Mixed Beads to each well.
   (**Note:** During addition of Mixed Beads, shake Mixing 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 4 °C.

9. Gently remove well contents and wash plate 2 times following instructions listed in the Plate Washing section.

10. Add 25 µL of Detection Antibodies into each well.
(**Note:** Allow the Detection Antibodies to warm to room temperature prior to addition.)

11. Seal, cover with foil, and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25 °C). **DO NOT ASPIRATE AFTER INCUBATION.**

12. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.
(**Note:** Allow the SAPE to warm to room temperature prior to addition.)

13. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25 °C).

14. Gently remove well contents and wash plate 2 times following instructions listed in the Plate Washing section.

15. Add 100 µL of Sheath Fluid PLUS (or Drive Fluid PLUS if using MAGPIX®) to all wells. Resuspend the beads by incubating with agitation on a plate shaker for 5 minutes at room temperature.

16. Run plate on Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® instrument with xPONENT® software or xMAP® INTELLIFLEX instrument with INTELLIFLEX software.

17. Save and analyze the Median Fluorescent Intensity (MFI) data using the 5-parameter logistic or spline curve-fitting method to calculate analyte concentrations in samples.

Remember to multiply the sample dilution factor for final sample results.

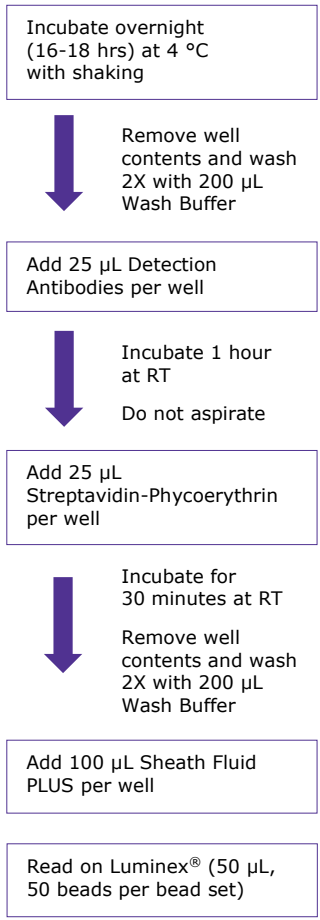


Plate Washing

Solid Plate

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

- Handheld magnet (Cat. 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 (Cat. No. 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.

Filter Plate (Cat. No. MX-PLATE)

If using a filter plate, use a vacuum filtration manifold to remove well contents. Wash plate with 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration after each wash. Repeat wash steps as recommended in the Assay Procedure.

Equipment Settings

Luminex® 200™, HTS, 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™ and HTS	xPONENT® 3.1 compatible Calibration Kit (Cat. No. LX2R-CAL-K25)	Performance Verification Kit (Cat. No. LX2R-PVER-K25)
FLEXMAP 3D®	FLEXMAP 3D® Calibrator Kit (Cat. No. F3D-CAL-K25)	FLEXMAP 3D® Performance Verification Kit (Cat. No. F3D-PVER-K25)
xMAP® INTELLIFLEX	xMAP® INTELLIFLEX Calibration Kit (Cat. No. IFX-CAL-K20)	xMAP® INTELLIFLEX Performance Verification Kit (Cat. No. IFX-PVER-K20)
MAGPIX®	MAGPIX® Calibration Kit (Cat. No. MPX-CAL-K25)	MAGPIX® Performance Verification Kit (Cat. 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 Cat. No. MAG-PLATE, if additional plates are required for this purpose.

Events	50, per bead	
Sample Size	50 µL	
Gate Settings	8,000 to 15,000	
Time Out	60 seconds	
Bead Set	13-plex Customizable Beads	
	sCD30 Bead	13
	sgp130	15
	sIL-1RI	25
	sIL-1RII	28
	sIL-2Rα	39
	sIL-4R	43
	sIL-6R	45
	sRAGE	47
	sTNFRI	62
	sTNFRII	66
	sVEGFR1	72
	sVEGFR2	75
	sVEGFR3	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

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.

	2 hour protocol, n=3 Assays	2 hour protocol	overnight protocol, n=5 Assays	overnight protocol
Analyte	**MinDC (pg/mL)**	**MinDC + 2SD (pg/mL)**	**MinDC (pg/mL)**	**MinDC + 2SD (pg/mL)**
sCD30	7	18.7	5	13.5
sgp130	38	74.3	15	22.0
sIL-1RI	2	4.6	3	6.6
sIL-1RII	3	7.1	4	6.4
sIL-2Rα	3	7.2	3	6.8
sIL-4R	4	4.3	2	5.7
sIL-6R	9	24.1	5	15.2
sRAGE	7	7.8	7	15.0
sTNFRI	5	10.1	6	11.8
sTNFRII	3	4.8	2	3.3
sVEGFR1	8	17.0	8	21.0
sVEGFR2	6	7.2	5	9.2
sVEGFR3	14	33.4	7	12.8

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 from across two different concentrations of analytes across 4 different assays.

Analyte	Intra-Assay (CV%)	Inter-Assay (CV%)
sCD30	4.9	10.6
sgp130	4.3	7.0
sIL-1RI	3.9	7.6
sIL-1RII	4.3	8.3
sIL-2Rα	5.2	10.9
sIL-4R	4.0	6.1
sIL-6R	5.8	9.2
sRAGE	5.7	11.4
sTNFRI	3.7	8.5
sTNFRII	3.6	6.2
sVEGFR1	5.9	9.6
sVEGFR2	4.9	9.4
sVEGFR3	5.8	8.8

Accuracy

Spike Recovery: The data represent mean percent recovery of three levels of spiked standards in diluted serum matrix.

Analyte	% Recovery in Matrix
sCD30	94
sgp130	89
sIL-1RI	98
sIL-1RII	97
sIL-2Rα	94
sIL-4R	91
sIL-6R	95
sRAGE	96
sTNFRI	96
sTNFRII	96
sVEGFR1	89
sVEGFR2	97
sVEGFR3	96

Cross-Reactivity

There was no or negligible cross-reactivity between the antibodies for an analyte and any of the other analytes in this panel.

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 flush, back flush and washes; or if needed probe should be removed and sonicated.
	Probe height not adjusted correctly	When reading the assay on the Luminex® 200™ instrument, adjust probe height to the kit solid plate or to the recommended filter plates using 3 alignment discs. When reading the assay on the MAGPIX® instrument, adjust probe height to the kit solid plate or to the recommended filter plates using 2 alignment discs. When reading the assay on the FLEXMAP 3D® instrument, adjust probe height to the kit solid plate using 1 alignment disc. For the FLEXMAP 3D® instrument, 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 the xMAP® INTELLIFLEX instrument, 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®.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and pipetting with multichannel pipets 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.

Problem	Probable Cause	Solution
Beads not in region or gate	Luminex® not calibrated correctly or recently	Calibrate Luminex® based on Instrument 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, Bioplex®) 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® 4 times to rid of air bubbles, wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.
	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.
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® Instrument (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.

Problem	Probable Cause	Solution
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 tech support for appropriate protocol modifications.
	Samples contain analyte concentrations higher than highest standard point.	Samples may require dilution and reanalysis for just that particular analyte.
	Standard curve was saturated at higher end of curve.	See above.
High variation in samples and/or standards	Multichannel pipet may not be calibrated	Calibrate pipets.
	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 a vertical 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 pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.

FOR FILTER PLATES ONLY

Problem	Probable Cause	Solution
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

Replacement Reagents	Cat. No.
Mouse Soluble Cytokine Receptor Standard	MSCR8042
Mouse Soluble Cytokine Receptor Quality Controls 1 and 2	MSCR6042
Mouse Soluble Cytokine Receptor Detection Antibodies	MSCR1042
Serum Matrix	LMC-SD
Streptavidin-Phycoerythrin	L-SAPE3
Assay Buffer	L-AB
Set of two 96-Well Plates with Sealers	MAG-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Magnetic Beads

Analyte	Bead No.	Cat. No.
sCD30	13	MSCD30-MAG
sgp130	15	MSGP130-MAG
sIL-1RI	25	MSIL1R1-MAG
sIL-1RII	28	MSIL1R2-MAG
sIL-2Rα	39	MSIL2RA-MAG
sIL-4R	43	MSIL4R-MAG
sIL-6R	45	MSIL6R-MAG
sRAGE	47	MSRAGE-MAG
sTNFRI	62	MSTNFR1-MAG
sTNFRII	66	MSTNFR2-MAG
sVEGFR1	72	MSVEGFR1-MAG
sVEGFR2	75	MSVEGFR2-MAG
sVEGFR3	77	MSVEGFR3-MAG

Well Map

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