Mouse Soluble Cytokine Receptor Magnetic Bead Panel

96-Well Plate Assay

Cat. # MSCRMAG-42K

MILLIPLEX® MAP

MOUSE SOLUBLE CYTOKINE RECEPTOR MAGNETIC BEAD KIT
96-Well Plate Assay

MSCRMAG-42K

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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.

To identify specific cytokines and/or soluble cytokine receptors that are involved in normal or disease immune response, it might be necessary to screen large panels, often requiring some level of automation and/or high throughput. Magnetic Beads can make the process of automation and high throughput screening easier with features such as walk-away washing. Advantages outside automation include:

- More flexible plate and plate washer options
- Improved performance with turbid serum/plasma samples
- Assay results equivalent to non-magnetic beads
- Automated washing eliminates technical obstacles (i.e., clogging of wells that contain viscous samples) which may result during vacuum manifold/manual washing
- Designed for the simultaneous analysis of multiple biomarkers, the Mouse Soluble Cytokine Receptor Panel provides important tools for the study of inflammatory and immune responses.

Therefore, the MILLIPLEX® MAP Mouse Soluble Cytokine Receptor panel 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 multiple analytes simultaneously, which can dramatically improve productivity. Millipore's MILLIPLEX® MAP Mouse Soluble Cytokine panel is the most versatile system available for cytokine receptor research. MILLIPLEX® MAP offers you the ability to:

- Select a 13-plex pre-mixed kit or
- 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 gives you the assurance that you will have all the necessary reagents you need to run your assay

EMD Millipore's MILLIPLEX® MAP Mouse Soluble Cytokine Receptor Panel kit can be used for the simultaneous quantification of the following 13 Mouse Cytokines: 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® MAP is based on the Luminex® xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life sciences and capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded beads known as MagPlex™-C microspheres.

- Luminex uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, 100 distinctly colored bead sets 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-Phycoerythrin (SA-PE) conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The microspheres are allowed to pass rapidly through a laser that excites the internal dyes marking the microsphere set. A second laser excites PE, the fluorescent dye on the reporter molecule.
- Finally, high-speed digital-signal processors identify each individual microsphere and quantify the result of its bioassay based on fluorescent reporter signals.

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

Note: Store all reagents at 2 – 8°C

REAGENTS SUPPLIED	CATALOG NUMBER	VOLUME	QUANTITY
Mouse Soluble Cytokine Receptor Standard	MSCR8042	Lyophilized	1 vial
Mouse Soluble Cytokine Receptor Quality Controls 1 and 2	MSCR6042	Lyophilized	2 vials
Mouse Soluble Cytokine Receptor Detection Antibodies	MSCR1042	3.2 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE3	3.2 mL	1 bottle
Serum Matrix Note: Contains 0.08% Sodium Azide	LMC-SD	Lyophilized	1 vial
Assay Buffer Note: Contains 0.08% Sodium Azide	L-AB	30 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	2 bottles
Set of one 96-Well Microtiter Plate with 2 Sealers	----------	----------	1 plate 2 sealers
Mixing Bottle	----------	----------	1 bottle

Included Mouse Soluble Cytokine Receptor Antibody-Immobilized Beads are dependent on customizable selection of analytes within the panel (see following table on page 5).

Mouse Soluble Cytokine Receptor Antibody-Immobilized Beads:

Bead/Analyte Name	Luminex Bead Region	Customizable 13 Analytes (20X concentration, 200 µL)	
		Available	Cat. #
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 BUT NOT PROVIDED

Reagents

1. Luminex Sheath Fluid (Luminex Catalog #40-50000) or Luminex Drive Fluid (Luminex Catalog # MPXDF-4PK)

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
2. Multichannel Pipettes capable of delivering 5 µL to 50 µL or 25 µL to 200 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Aluminum Foil
7. Absorbent Pads
8. Laboratory Vortex Mixer
9. Sonicator (Branson Ultrasonic Cleaner Model #B200 or equivalent)
10. Titer Plate Shaker (Lab-Line Instruments Model #4625 or equivalent)
11. Luminex 200™, HTS, FLEXMAP 3D™, or MAGPIX® with xPONENT® software by Luminex Corporation
12. Automatic Plate washer for magnetic beads (Bio-Tek ELx405, EMD Millipore Catalog #40-015 or equivalent) or Hand-held Magnetic Separation Block (EMD Millipore Catalog #40-285 or equivalent)

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

SAFETY PRECAUTIONS

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

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 Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 4 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. When reading the assay on MAGPIX, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 2 alignment discs.
- 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

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- 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..

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anticoagulant is recommended. Centrifuge for 10 minutes at 1000xg 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..

o 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

A. 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.

B. Preparation of Quality Controls

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

C. Preparation of Wash Buffer

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

D. Preparation of Serum Matrix

This step is required for serum or plasma samples only.

Add 1.0 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 REAGENTS FOR IMMUNOASSAY (continued)

E. 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.

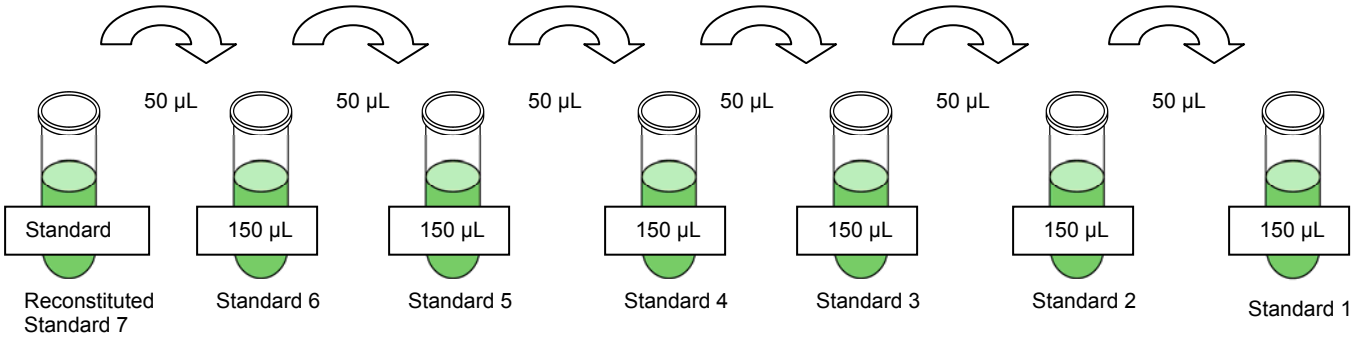
2.) Preparation of Working Standards

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	Volume of Deionized Water to Add	Volume of Standard to Add
Standard 7 (Reconstituted Standard)	250 µL	0

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

Preparation of Standards



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

Standard Tube #	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.)

Add 200 µL Wash Buffer per well

Shake 10 min, RT

Decant

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

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 w**ell contents a**nd wash p**l**ate 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 (or Drive Fluid if using MAGPIX®) to all wells. Resuspend the beads by incubating with agitation on a plate shaker for 5 minutes at room temperature.

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

17. Save and analyze the Median Fluorescent Intensitity (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.

Incubate overnight (16-18 hrs) at 4°C with shaking

↓

Remove well contents and wash 2X with 200 µL Wash Buffer

Add 25 µL Detection Antibodies per well

↓

Incubate 1 hour at RT

Do not aspirate

Add 25 µL Streptavidin-Phycoerythrin per well

↓

Incubate for 30 minutes at RT

Remove well contents and wash 2X with 200 µL Wash Buffer

Add 100 µL Sheath Fluid per well

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

PLATE WASHING

1.) Solid Plate

If using a solid plate, use either a hand-held magnet or magnetic plate washer.

A.) For hand-held magnet, 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 uL of Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps as recommended in Assay Procedure.

B.) For magnetic plate washer, let plate "soak" on magnet for 60 seconds to allow complete settling of the magnetic beads. Remove well contents by aspiration. Wash plate with 200 µL/well of Wash Buffer, letting beads "soak" for 60 seconds and removing Wash Buffer by aspiration after each wash. Repeat wash steps as recommended in Assay Procedure. **Note:** If using the recommended plate washer for magnetic beads (Bio-Tek ELx405) follow the appropriate equipment settings outlined in **EQUIPMENT SETTINGS.**

2.) Filter Plate (EMD Millipore Cat #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

Bio-Tek ELx405:
The general recommended wash protocol (Link Protocol) is as follows:

Soak Program:	Wash Program:
Soak →	Aspirate→Dispense→Soak→Aspirate→Dispense→Soak→Aspirate

1.) Soak program:

1. Soak duration: 60 sec
2. Shake before soak?: NO

2.) Wash program:

Method:

1. Number of cycles: 2
2. Soak/shake: YES
3. Soak duration: 60 sec
4. Shake before soak: NO
5. Prime after soak: NO

EQUIPMENT SETTINGS (continued)

Dispense:

1. Dispense volume: 200 µL/well
2. Dispense flow rate: 5
3. Dispense height: 130 (16.51 mm)
4. Horizontal disp pos: 00 (0 mm)
5. Disable Aspirate: YES
6. Bottom Wash first?: NO
7. Prime before start?: NO

Aspiration:

1. Aspirate height: 35 (4.445 mm)
2. Horizontal Asp Pos: 30 (1.372 mm)
3. Aspiration rate: 06 (15.0 mm/sec)
4. Aspiration delay: 0
5. Crosswise Aspir: NO
6. Final Aspir: YES
7. Final Aspir delay: 0 (0 msec)

3.) Link program: (**Note:** this is the program to use during actual plate washing). Link together the Soak and Wash programs outlined above.

Note: 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 ELx405, please refer to the manufacturer's recommendations for programming instructions.

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

These specifications are for the Luminex 200™, Luminex HTS, Luminex FLEXMAP 3D® and Luminex MAGPIX®with xPONENT® software. Luminex instruments with other software (e.g. MasterPlex, StarStation, LiquiChip, Bio-Plex, LABScan100) would need to follow instrument instructions for gate settings and additional specifications from the vendors for reading Luminex Magnetic Beads.

For magnetic bead assays, the Luminex 200™ and HTS instruments must be calibrated with the xPONENT® 3.1 compatible Calibration Kit (EMD Millipore Cat #40-275) and performance verified with the Performance Verification Kit (EMD Millipore Cat #40-276). The Luminex FLEXMAP 3D® instrument must be calibrated with the FLEXMAP 3D® Calibrator Kit (EMD Millipore Cat #40-028) and performance verified with the FLEXMAP 3D® Performance Verification Kit (EMD Millipore Cat #40-029). The Luminex MAGPIX® instrument must be calibrated with the MAGPIX® Calibration Kit (EMD Millipore Cat #40-049) and performance verified with the MAGPIX® Performance Verification Kit (EMD Millipore Cat #40-050).

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

EQUIPMENT SETTINGS (continued)

The Luminex probe height must be adjusted to the plate provided in the kit. Please use Cat #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 the EMD MILLIPORE website www.millipore.com/techlibrary/index.do using the catalog number as the keyword.

ASSAY CHARACTERISTICS

Assay Sensitivities (minimum detectable concentrations, pg/mL)

MinDC: Minimum Detectable Concentration is calculated using MILLIPLEX® Analyst. 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	*2 hour protocol, n=3 Assays*	*2 hour protocol*	*overnight protocol, n=5 Assays*	*overnight protocol*
	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

ASSAY CHARACTERISTICS (continued)

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 GUIDE

Problem	Probable Cause	Solution
Insufficient Bead Count	Plate Washer aspirate height set too low	Adjust aspiration height according to manufacturers' instructions.
	Bead mix prepared inappropriately	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting this into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with Alcohol flush, Back flush 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 according to the protocols recommended by Luminex to the kit solid plate using 4 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. When reading the assay on MAGPIX, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 2 alignment discs.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately, and pipeting with Multichannel pipets without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for cross reacting components (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes.
Beads not in region or gate	Luminex 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^{o}C.
	Gate Settings not adjusted correctly	Some Luminex instruments (e.g. 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	Keep plate and bead mix covered with dark

	light	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 (e.g. Bio-plex) Default target setting for RP1 calibrator is set at High PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with standard curve and samples	Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Check with 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	
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.

REPLACEMENT REAGENTS

Components	Cat #
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 Beads

Soluble Cytokine Receptor	Bead #	Cat. #
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

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Include:

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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									