

User Guide

MILLIPLEX® Human Cytokine Autoantibody IgM Magnetic Bead Panel

96-Well Plate Assay

HCYTABM-17K

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Introduction

Anti-cytokine antibodies occur frequently and are present in healthy individuals and patients with acquired immunodeficiency and autoimmune diseases. Cytokines offering protection against microbes can be targeted by cytokine autoantibodies, leading to life-threatening infections. Some infections associated with anti-cytokine antibodies include *M. tuberculosis*, *Salmonella*, *S. aureus*, *E. coli*, *Histoplasma*, and more. The presence of cytokine autoantibodies in autoimmune disease can influence disease severity and activity. These autoimmune diseases may include systemic lupus erythematosus, multiple sclerosis, Sjögren's syndrome, rheumatoid arthritis, and acute respiratory distress syndrome. Measuring cytokine autoantibodies may be useful for disease monitoring and efficacy of treatment.

MILLIPLEX® products offer 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).

In addition, each panel and kit meets stringent manufacturing criteria to ensure batch-to-batch reproducibility. The MILLIPLEX® Human Cytokine Autoantibody IgM Magnetic Bead Panel thus enables you to focus on the therapeutic potential of protein biomarkers for liver toxicity. Coupled with the Luminex® xMAP® platform in a magnetic bead format, you receive the advantage of ideal speed and sensitivity, allowing qualitative multiplex detection of dozens of analytes simultaneously, which can dramatically improve productivity.

The MILLIPLEX® Human Cytokine Autoantibody IgM Magnetic Bead Panel is part of the most versatile system available for cytokine autoantibody 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 antibody detection.

MILLIPLEX® products offer you:

- The ability to choose any combination of analytes from our panel of 23 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® Human Cytokine Autoantibody IgM Magnetic Bead Panel is a 23-plex kit to be used for the simultaneous detection of any or all of the following analytes in serum and plasma samples: Anti-BAFF, Anti-G-CSF, Anti-IFN β , Anti-IFN γ , Anti-IL-1 α , Anti-IL-6, Anti IL 8, Anti-IL-10, Anti-IL-12p40, Anti-IL-15, Anti-IL-17A, Anti-IL-17F, Anti-IL-18, Anti-IL-22, Anti-TNF α , Anti-IFN α 2, Anti-GM-CSF, Anti-IL-2, Anti-IL-3, Anti-IL-4, Anti-Osteopontin, Anti-PF4, and Anti-IFN ω .

For this panel, 4 assay control beads are included as part of the base format.

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 antigen.
- After an antibody from a test sample is captured by the bead, the PE-IgM conjugate is introduced 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 qualified based on fluorescent reporter signals. We combine the streamlined data acquisition power of Luminex® xPONENT® acquisition software with sophisticated analysis capabilities of the 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.
- **DO NOT** FREEZE Antigen-Immobilized Beads and PE-IgM Conjugate.

Reagents Supplied

Store all reagents at 2-8 °C

Reagents	Volume	Quantity	Cat. No.
Set of one 96-Well Plate with 2 sealers	-	1 set	-
Assay Buffer	30 mL	1 bottle	L-AB
10X Wash Buffer**	60 mL	1 bottle	L-WB
Human Cytokine Autoantibody IgM Panel PE-IgM Conjugate	5.5 mL	1 bottle	HCYTAB- PEIGM
Mixing Bottle	-	1 bottle	-

** Contains 0.05% Proclin

Human Cytokine Autoantibody IgM Control Antigen-Immobilized Magnetic Beads

Bead/Analyte Name	Luminex® Magnetic Bead Region	Control Beads (50X concentration, 90 µL)	
		Available	Cat. No.
Control 1 IgM Bead	12	✓	HAACB1M-MAG
Control 2 IgM Bead	13	✓	HAACB2M-MAG
Control 3 IgM Bead	14	✓	HAACB3M-MAG
Negative Control Bead	15	✓	NCB-MAG

**The 4 Control Beads are to be combined with
the 23 Antigen-Immobilized Beads to make up to a 27-plex.**

Included Human Cytokine Autoantibody IgM Antigen-Immobilized Beads are dependent on customizable selection of analytes within the panel.

Human Cytokine Autoantibody IgM Antigen-Immobilized Magnetic Beads

Bead/Analyte Name	Luminex® Magnetic Bead Region	Customizable 23 Analytes (50X concentration, 90 µL)	
		Available	Cat. No.
IFN ω Bead	18	✓	HAAIFN0-MAG
GM-CSF Bead	20	✓	HAAGMCSF-MAG
IL-2 Bead	22	✓	HAAIL2-MAG
IFN β Bead	25	✓	HAAIFNB-MAG
IL-22 Bead	30	✓	HAAIL22-MAG
IL-12p40 Bead	36	✓	HAAIL12P40-MAG
IL-3 Bead	39	✓	HAAIL3-MAG
IL-4 Bead	43	✓	HAAIL4-MAG
Osteopontin Bead	45	✓	HAAOPN-MAG
PF-4 Bead	48	✓	HAAPF4-MAG
IFN α 2 Bead	52	✓	HAAIFNA2-MAG
IL-6 Bead	54	✓	HAAIL6-MAG
IL-15 Bead	56	✓	HAAIL15-MAG
IL-17A Bead	62	✓	HAAIL17A-MAG
IL-17F Bead	64	✓	HAAIL17F-MAG
G-CSF Bead	65	✓	HAAGCSF-MAG
TNF α Bead	67	✓	HAATNFA-MAG
IL-10 Bead	72	✓	HAAIL10-MAG
BAFF Bead	73	✓	HAABAFF-MAG
IFN γ Bead	75	✓	HAAIFNG-MAG
IL-1 α Bead	76	✓	HAAIL1A-MAG
IL-8 Bead	77	✓	HAAIL8-MAG
IL-18 Bead	78	✓	HAAIL18-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 (Lab-Line Instruments Model No. 4625 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	
PE-IgM Conjugate	HCYTAB- PEIGM	 	Warning. Causes serious eye irritation. May cause damage to organs Respiratory Tract through prolonged or repeated exposure. Do not breathe dust/ fume/ gas/ mist/ vapors/ spray. Wash skin thoroughly after handling. Wear eye protection/ face protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. If eye irritation persists: Get medical advice/ attention. Dispose of contents/ container to an approved waste disposal plant.
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 Antigen-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.
- Any unused mixed Antigen-Immobilized Beads may be stored in the Mixing Bottle at 2-8 °C for up to one month.
- 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 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 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 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®.
- For serum/plasma samples that require further dilution beyond 1:100, use the Assay Buffer provided in the kit.
- Vortex all reagents well before adding to plate.

Sample Collection and Storage

Preparation of Serum Samples

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C.
- Avoid multiple (> 2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Serum samples should be diluted 1:100 in the Assay Buffer provided in the kit. For example, in a tube, 10 µL of serum may be combined with 90 µL of Assay Buffer to make a 1:10 dilution and then 10 µL of the 1:10 dilution may be combined with 90 µL of Assay Buffer to make the 1:100 dilution. When further dilution beyond 1:100 is required, use Assay Buffer as the diluent.

Preparation of Plasma Samples

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000 x g within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20 °C.
- Avoid multiple (> 2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Plasma samples should be diluted 1:100 in the Assay Buffer provided in the kit. For example, in a tube, 10 µL of plasma may be combined with 90 µL of Assay Buffer and then 10 µL of the 1:10 dilution may be combined with 90 µL of Assay Buffer to make the 1:100 dilution. When further dilution beyond 1:100 is required, use Assay Buffer as the diluent.

NOTE:

- A maximum of 25 µL per well of diluted serum or plasma can be used.

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

Preparation of Reagents for Immunoassay

Preparation of Antigen-Immobilized Beads

For individual vials of beads, sonicate each antigen-bead vial for 30 seconds; vortex for 1 minute. Add 60 μ L from each antigen-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 10 antigen-immobilized beads and the 4 control beads, add 60 μ L from each of the 14 bead vials to the Mixing Bottle. Then add 2.16 mL Assay Buffer.

Example 2: When using 15 antigen-immobilized beads and 4 control beads, add 60 μ L from each of the 19 bead vials to the Mixing Bottle. Then add 1.86 mL Assay Buffer.

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 (one bottle) with 540 mL deionized water. Store the unused portion at 2-8 °C for up to one month.

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 and Samples on Well Map Worksheet in a vertical configuration.

Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.

- 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 μ L 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 μ L of Assay Buffer to all wells.
4. Add 25 μ L of Sample (diluted) into the appropriate wells.
5. Add an additional 25 μ L of Assay Buffer to the background wells.
6. Vortex Mixing Bottle and add 25 μ L of the Mixed 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 μ L Wash Buffer per well



Shake 10 min, RT
Decant

- Add 25 μ L Assay Buffer to all wells
- Add 25 μ L diluted Samples to sample wells
- Add an additional 25 μ L of Assay Buffer to background wells
- Add 25 μ L 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 μ L of Human Cytokine Autoantibody PE-IgM Conjugate into each well.
(**Note:** Allow the PE-IgM Conjugate to warm to room temperature prior to addition.)
10. Seal, cover with foil and incubate with agitation on a plate shaker for 90 minutes at room temperature (20-25 $^{\circ}$ C).
11. Gently remove well contents and wash plate 3 times following instructions listed in the Plate Washing section.
12. Add 150 μ L of Sheath Fluid PLUS (or Drive Fluid PLUS if using MAGPIX[®]) to all wells. Resuspend the beads on a plate shaker for 5 minutes.
13. Run plate on Luminex[®] 200™, HTS, FLEXMAP 3D[®], MAGPIX[®] instrument with xPONENT[®] software or xMAP[®] INTELLIFLEX instrument with INTELLIFLEX software.
14. Save and analyze the Median Fluorescent Intensity (MFI) data. A working cut-off for a positive result can be established using MFI data from known negative sample population.



Remove well contents and wash 3X with 200 μ L Wash Buffer

Add 50 μ L PE-IgM Conjugate per well



Incubate 90 minutes at RT

Remove well contents and wash 3X with 200 μ L Wash Buffer

Add 150 μ L Sheath Fluid PLUS or Drive Fluid PLUS per well

Read on Luminex[®] instrument (100 μ L, 50 beads per bead set)

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 μ L 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 μ L 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 μ L/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® instruments with xPONENT® software and xMAP® INTELLIFLEX instrument 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	100 µL
Gate Settings	8,000 to 15,000
Reporter Gain	Default (low PMT)
Time Out	60 seconds
Bead Set	Customizable 23-plex Beads + Control Beads
	Control IgA 1 12
	Control IgA 2 13
	Control IgA 3 14
	Negative Control 15
	IFNω 18
	GM-CSF 20
	IL-2 22

Bead Set	Customizable 23-plex Beads + Control Beads	
	IFN β	25
	IL-22	30
	IL-12p40	36
	IL-3	39
	IL-4	43
	Osteopontin	45
	PF-4	48
	IFN α 2	52
	IL-6	54
	IL-15	56
	IL-17A	62
	IL-17F	64
	G-CSF	65
	TNF α	67
	IL-10	72
	BAFF	73
	IFN γ	75
	IL-1 α	76
	IL-8	77
	IL-18	78

Assay Characteristics

Precision

Intra-assay precision is <15% for this assay generated from the mean of the % CV's from 8 reportable results in a single assay. Inter-assay precision is <20% for this assay generated from the mean of the %CV's across 3 different assays.

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 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. For 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 pipettes without touching reagent in plate.
	Insufficient washes	Increase number of washes.

Problem	Probable Cause	Solution
Beads not in region or gate	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.
	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.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex® instrument 4 times to rid it of air bubbles, wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.
Signal for whole plate is same as background	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.
	Incorrect or no PE-IgM conjugate was added	Add PE-IgM conjugate according to protocol. If PE-IgM Conjugate has already been removed, sensitivity may be low.
	Multichannel pipette may not be calibrated	Calibrate pipettes.
High variation in samples	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.

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.

Plate Ordering

Replacement Reagents	Cat. No.
Assay Buffer	L-AB
Human Cytokine Autoantibody PE-IgM Conjugate	HCYTAB-PEIGM
Control IgM Bead 1	HAACB1M-MAG
Control IgM Bead 2	HAACB2M-MAG
Control IgM Bead 3	HAACB3M-MAG
Negative Control Bead	NCB-MAG
Set of two 96-Well plates with sealers	MAG-PLATE
10X Wash Buffer	L-WB

Antigen-Immobilized Magnetic Beads

Analyte	Bead No.	Cat. No.	Analyte	Bead No.	Cat. No.
IFN ω	18	HAAIFN0-MAG	IL-15	56	HAAIL15-MAG
GM-CSF	20	HAAGMCSF-MAG	IL-17A	62	HAAIL17A-MAG
IL-2	22	HAAIL2-MAG	IL-17F	64	HAAIL17F-MAG
IFN β	25	HAAIFNB-MAG	G-CSF	65	HAAGCSF-MAG
IL-22	30	HAAIL22-MAG	TNF α	67	HAATNFA-MAG
IL-12p40	36	HAAIL12P40-MAG	IL-10	72	HAAIL10-MAG
IL-3	39	HAAIL3-MAG	BAFF	73	HAABAFF-MAG
IL-4	43	HAAIL4-MAG	IFN γ	75	HAAIFNG-MAG
Osteo-pontin	45	HAA0PN-MAG	IL-1 α	76	HAAIL1A-MAG
PF-4	48	HAAPF4-MAG	IL-8	77	HAAIL8-MAG
IFN α 2	52	HAAIFNA2-MAG	IL-18	78	HAAIL18-MAG
IL-6	54	HAAIL6-MAG			

Well Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Back-ground											
B	Back-ground											
C	Sample 1											
D	Sample 1											
E	Sample 2											
F	Sample 2											
G	Etc.											
H												

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