Human Cytokine Autoantibody Magnetic Bead Panel

96-Well Plate Assay

Cat. # HCYTAAB-17K

MILLIPLEX® MAP

HUMAN CYTOKINE AUTOANTIBODY MAGNETIC BEAD PANEL
96-Well Plate Assay

HCYTAAB-17K

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

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex® Corporation ("Luminex®"), you, the customer, acquire the right under Luminex®'s patent rights, if any, to use this product or any portion of this product, including without limitation the microsphere beads contained herein, only with Luminex®'s laser based fluorescent analytical test instrumentation marketed under the name of Luminex® 100™ IS, 200™, HTS, FLEXMAP 3D®, MAGPIX®.

INTRODUCTION

Anti-cytokine antibodies occur frequently and are present in healthy individuals and patients with acquired immunodeficiency and autoimmune dieases. 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® MAP 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 validation process include: cross-reactivity, dilution linearity, kit stability, and sample behavior (e.g. detectability and stability).

In addition each panel and kit meets stringent manufacturing criteria to ensure batch-to-batch reproducibility. The MILLIPLEX® MAP Human Cytokine Autoantibody Magnetic Bead Panel thus enables you to focus on the therapeutic potential of Cytokine Autoantibodies. 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.

EMD Millipore's MILLIPLEX® MAP Human Cytokine Autoantibody 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 validate and build the most comprehensive library available for protein detection and quantitation.

- MILLIPLEX® MAP offers you:
    - The ability to choose any combination of analytes from our panel of 15 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.

EMD Millipore's MILLIPLEX® MAP Human Cytokine Autoantibody Magnetic Bead Panel is a 19-plex kit to be used for the simultaneous quantification of any or all of the following analytes in serum and plasma samples: Anti-B-Cell Activating Factor (Anti-BAFF), Anti-Granulocyte-Colony Stimulating Factor (Anti-G-CSF), Anti-Interferon β (Anti-IFNβ), Anti-Interferon γ (Anti-IFNγ), Anti-Interleukin-1α (Anti-IL-1α), Anti-Interleukin-6 (Anti-IL-6), Anti-Interleukin-8 (Anti-IL-8), Anti-Interleukin-10 (Anti-IL-10), Anti-Interleukin-12p40 (Anti-IL-12p40), Anti-Interleukin-15 (Anti-IL-15), Anti-Interleukin-17A (Anti-IL-17A), Anti-Interleukin-17F (Anti-IL-17F), Anti-Interleukin-18 (Anti-IL-18), Anti-Interleukin-22 (Anti-IL-22), and Anti-Tumor Necrosis Factor Alpha (Anti-TNFα).

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

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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 magnetic 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, 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 antigen.
- After an antibody from a test sample is captured by the bead, the PE-IgG conjugate is introduced to complete the reaction on the surface of each microsphere.
- The reaction mixture is then incubated with PE-IgG conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- EMD Millipore provides three Luminex® instruments to acquire and analyze data using two detection methods:
    - The Luminex® analyzers Luminex® 200™ and FLEXMAP 3D®, 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. EMD Millipore combines 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.

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 or PE-IgG Conjugate**

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8°C

Reagents Supplied	Catalog Number	Volume	Quantity
Set of one 96-Well Plate with 2 sealers	----------	----------	1 plate 2 sealers
Assay Buffer	L-AB	30 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	60 mL	1 bottle
Human Cytokine Autoantibody Panel PE-IgG Conjugate	HCYTAA-PEIGG	5.5 mL	1 bottle
Mixing Bottle	----------	----------	1 bottle

Control Antigen-Immobilized Magnetic Beads:

Bead Name	Luminex® Magnetic Bead Region	Control Beads (50X concentration, 90 µL) Available	Cat. #
Control 1 Beads Magnetic	12	✔	CB1-MAG
Control 2 Beads Magnetic	13	✔	CB2-MAG
Control 3 Beads Magnetic	14	✔	CB3-MAG
Negative Control Beads Magnetic	15	✔	NCB-MAG

The 4 Control Beads are to be combined with the 15 Antigen-Immobilized Beads to make up to a 19-plex.

Included Human Cytokine Autoantibody Antigen-Immobilized Beads are dependent on customizable selection of analytes within the panel (see next page).

Human Cytokine Autoantibody Antigen-Immobilized Magnetic Beads:

Bead/Antigen Name	Luminex® Magnetic Bead Region	Customizable 15 Antigen (50X concentration, 90 µL) Available	Cat. #
Human IFNβ Beads Magnetic	25	✔	HAAIFNB-MAG
Human IL-22 Beads Magnetic	30	✔	HAAIL22-MAG
Human IL-12p40 Beads Magnetic	36	✔	HAAIL12P40-MAG
Human IL-6 Beads Magnetic	54	✔	HAAIL6-MAG
Human IL-15 Beads Magnetic	56	✔	HAAIL15-MAG
Human IL-17A Beads Magnetic	62	✔	HAAIL17A-MAG
Human IL-17F Beads Magnetic	64	✔	HAAIL17F-MAG
Human G-CSF Beads Magnetic	65	✔	HAAGCSF-MAG
Human TNFα Beads Magnetic	67	✔	HAATNFA-MAG
Human IL-10 Beads Magnetic	72	✔	HAAIL10-MAG
Human BAFF Beads Magnetic	73	✔	HAABAFF-MAG
Human IFNγ Beads Magnetic	75	✔	HAAIFNG-MAG
Human IL-1α Beads Magnetic	76	✔	HAAIL1A-MAG
Human IL-8 Beads Magnetic	77	✔	HAAIL8-MAG
Human IL-18 Beads Magnetic	78	✔	HAAIL18-MAG

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex® Sheath Fluid (EMD Millipore Catalog # 40-50015) or Luminex® Drive Fluid (EMD Millipore 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 (VWR® Microplate Shaker Cat # 12620-926 or equivalent)
11. Luminex® 200™, HTS, FLEXMAP 3D®, or MAGPIX® with xPONENT® software by Luminex® Corporation
12. Automatic Plate Washer for magnetic beads (BioTek® 405 LS and 405 TS, EMD Millipore Catalog #40-094, # 40-095, # 40-096, # 40-097 or equivalent) or Handheld Magnetic Separation Block (EMD Millipore Catalog # 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 (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.

Full Hazard Label:

Ingredient, Cat #		Full Label	
PE-IgG Conjugate	HCYTAA-PEIGG		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
10X Wash Buffer	L-WB		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

TECHNICAL GUIDELINES

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- 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**.**
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes.
- When reading the assay on Luminex® 200™, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended EMD Millipore filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended EMD Millipore filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate using 1 alignment disc.

  For FLEXMAP 3D® when using the solid plate in the kit, the final resuspension should be with 150 µL Sheath Fluid in each well and 75 µL should be aspirated.
- 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

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.
- 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 1:100 dilution . When further dilution beyond 1:100 is required, use Assay Buffer as the diluent.
- When further dilution beyond 1:100 is required, use Assay Buffer as the diluent.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000xg 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 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 1:100 dilution . When further dilution beyond 1:100 is required, use Assay Buffer as the diluent.
- 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

A. Preparation of Antibody-Immobilized Beads

For individual vials of beads, sonicate each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 60 µL from each antibody-bead vial to the Mixing Bottle and bring final volume to 3.0 mL with 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 µL from each of the 14 bead vials to the Mixing Bottle. Then add 2.16 mL of Assay Buffer.

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

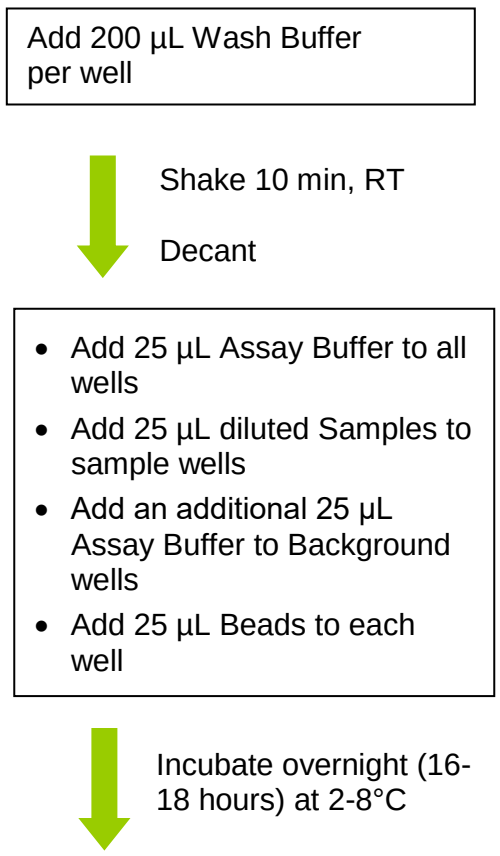
B. Preparation of Wash Buffer

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

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 the Background and Samples wells 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 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 Assay Buffer to all wells.
4. Add 25 µL of Sample (diluted) into the appropriate wells.
5. Add an additional 25 µL of Assay Buffer to background wells.
6. Vortex Mixing Bottle and add 25 µL of the Mixed 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. Alternatively, incubate for 2 hours at room temperature (20-25°C).



8. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
9. Add 50 µL of Human Cytokine Autoantibody PE-IgG Conjugate into each well.

   (Note: Allow the PE-IgG 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°C).
11. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
12. Add 150 µL of Sheath Fluid (or Drive Fluid 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® or MAGPIX® with xPONENT® 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.

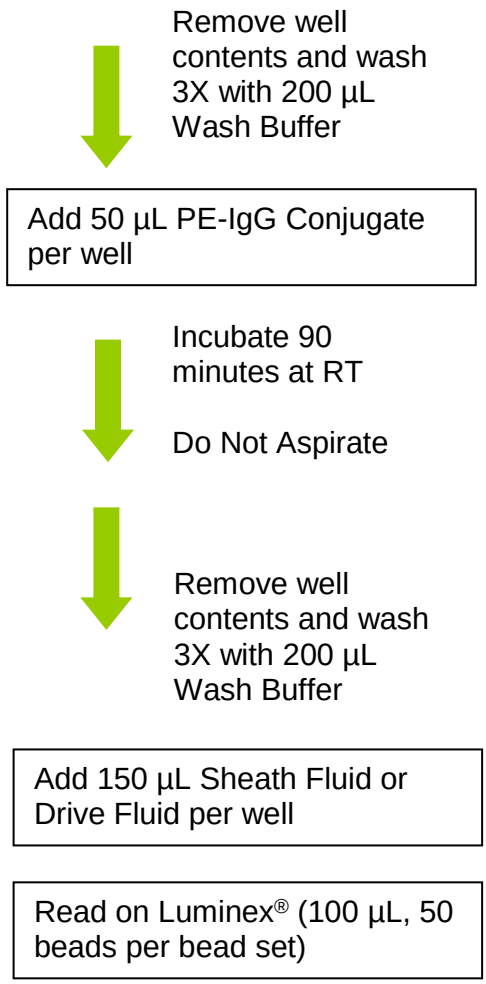


PLATE WASHING

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

1.) Solid Plate

A.) Handheld magnet **(EMD Millipore Catalog #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.

B.) Magnetic plate washer **(EMD Millipore Catalog #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.

2.) Filter Plate (EMD Millipore Catalog #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®, 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® 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, the Luminex® 200™ and HTS instruments must be calibrated with the xPONENT® 3.1 compatible Calibration Kit (EMD Millipore Catalog # LX2R-CAL-K25)) and performance verified with the Performance Verification Kit (EMD Millipore Catalog # LX2R-PVER-K25). The Luminex® FLEXMAP 3D® instrument must be calibrated with the FLEXMAP 3D® Calibrator Kit (EMD Millipore Catalog # F3D-CAL-K25) and performance verified with the FLEXMAP 3D® Performance Verification Kit (EMD Millipore Catalog # F3D-PVER-K25). The Luminex® MAGPIX® instrument must be calibrated with the MAGPIX® Calibration Kit (EMD Millipore Catalog # MPX-CAL-K25) and performance verified with the MAGPIX® Performance Verification Kit (EMD Millipore Catalog # 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.

EQUIPMENT SETTINGS (continued)

The Luminex® probe height must be adjusted to the plate provided in the kit. Please use Catalog #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 19-plex Beads	
	Control Beads 1	12
	Control Beads 2	13
	Control Beads 3	14
	Negative Control Beads	15
	IFNβ Beads	25
	IL-22 Beads	30
	IL-12p40 Beads	36
	IL-6 Beads	54
	IL-15 Beads	56
	IL-17A Beads	62
	IL-17F Beads	64
	G-CSF Beads	65
	TNFα Beads	67
	IL-10 Beads	72
	BAFF Beads	73
	IFNγ Beads	75
	IL1α Beads	76
	IL-8 Beads	77
	IL-18 Beads	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 4 different assays.

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 flushes, back flushes and washes; or, if needed, probe should be removed and sonicated.
	Probe height not adjusted correctly	When reading the assay on Luminex® 200™, adjust probe height to the kit solid plate or to the recommended EMD Millipore filter plates using 3 alignment discs. When reading the assay on MAGPIX®, adjust probe height to the kit solid plate or to the recommended EMD Millipore filter plates using 2 alignment discs. When reading the assay on FLEXMAP 3D®, adjust probe height to the kit solid plate using 1 alignment disc. For FLEXMAP 3D® when using the solid plate in the kit, the final resuspension should be with 150 µL Sheath Fluid in each well and 75 µL should be aspirated.
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.
Beads not in region or gate	Luminex® instrument not calibrated correctly or recently	Calibrate Luminex® instrument based on manufacturer's instructions, at least once a week or if temperature has changed by >3°C.
	Gate settings not adjusted correctly	Some Luminex® instruments (e.g. Bio-Plex®) require different gate settings than those described in the kit protocol. Use instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex® instrument 4 times to rid it of air bubbles, wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.
	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.

Problem	Probable Cause	Solution
Signal for whole plate is same as background	PE-IgG Conjugate was not added	Add PE-IgG conjugate according to protocol. If PE-IgG conjugate has already been removed, sensitivity may be low.
High variation in samples	Multichannel pipette may not be calibrated	Calibrate pipettes.
	Plate washing was not uniform	Confirm all reagents are removed completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using an orbital plate shaker at a speed where beads are in constant motion without causing splashing.
	Cross-well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipette tips that are used for reagent additions and that pipette tip does not touch reagent in plate.

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.

REPLACEMENT REAGENTS	Catalog #
Assay Buffer	L-AB
Human Cytokine Autoantibody Panel PE-IgG Conjugate	HCYTAA-PEIGG
Control Bead 1 - Magnetic	CB1-MAG
Control Bead 2 - Magnetic	CB2-MAG
Control Bead 3 - Magnetic	CB3-MAG
Negative Control Bead - Magnetic	NCB-MAG
Set of two 96-Well plates with sealers	MAG-PLATE
10X Wash Buffer	L-WB

Antigen-Immobilized Magnetic Beads

Antigen	*Bead #*	*Cat. #*
IFNβ Beads Magnetic	25	HAAIFNB-MAG
IL-22 Beads Magnetic	30	HAAIL22-MAG
IL-12p40 Beads Magnetic	36	HAAIL12P40-MAG
IL-6 Beads Magnetic	54	HAAIL6-MAG
IL-15 Beads Magnetic	56	HAAIL15-MAG
IL-17A Beads Magnetic	62	HAAIL17A-MAG
IL-17F Beads Magnetic	64	HAAIL17F-MAG
G-CSF Beads Magnetic	65	HAAGCSF-MAG
TNFα Beads Magnetic	67	HAATNFA-MAG
IL-10 Beads Magnetic	72	HAAIL10-MAG
BAFF Beads Magnetic	73	HAABAFF-MAG
IFNγ Beads Magnetic	75	HAAIFNG-MAG
IL-1α Beads Magnetic	76	HAAIL1A-MAG
IL-8 Beads Magnetic	77	HAAIL8-MAG
IL-18 Beads Magnetic	78	HAAIL18-MAG

ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

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

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be downloaded through our website at emdmillipore.com/msds.

WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	(Background Well)											
B	(Background Well)											
C	Sample 1											
D	Sample 1											
E	Sample 2											
F	Sample 2											
G	Etc.											
H												