Human Immunoglobulin IgE Single Plex Magnetic Bead Kit

96 Well Plate Assay

Cat. # HGAMMAG-303E

MILLIPLEX® MAP

Human Immunoglobulin IgE Single-Plex Kit
96 Well Plate Assay

#HGAMMAG-303E

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

INTRODUCTION

Produced by plasma cells and lymphocytes, immunoglobulins (antibodies) are critically involved in immune response, attaching to antigens and playing a role in their destruction. Immunoglobulins (Ig) can be classified by isotype, classes that differ in function and antigen response due to structure variability. Five major isotypes have been identified in placental mammals: IgM, IgG, IgA, IgE and IgD (B-cell receptor) – all found in normal individuals. The isotype IgE is the least abundant immunoglobulin in plasma (IgE serum levels are in the 50-300ng/mL range compared to 10mg/ml for IgG). Diseases such as dermatitis, asthma, and parasitic infections trigger an increase of IgE levels. Other diseases such as autoimmune disorders, hepatitis, cancer, and malaria can lead to a reduction of IgE. Determining the level of IgE in serum can be very insightful for researchers studying these various diseases. In addition, identifying the IgE isotype can be a critical need during monoclonal antibody production as well as vaccine production.

Magnetic Beads can make the process of automation and high throughput screening easier with features such as walk-away washing. Beyond automation, other advantages of using magnetic bead technology include:

- More options for plate selections and plate washer
- Improved performance with turbid serum/plasma samples
- Elimination of technical obstacles associated with vacuum manifold/manual washing

With the combination of the Luminex xMAP® platform and the **magnetic bead** format, the researcher gains the advantage of ideal speed and sensitivity which can dramatically improve productivity.

Millipore's MILLIPLEX Human Immunoglobulin Isotyping Panel is to be used for the measurement of IgE in serum and tissue/cell lysate and culture supernatant samples.

This kit is for research purposes only.

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 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-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 which 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.
- Once the standards and controls have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE RECONSTITUTED STANDARDS OR CONTROLS IN GLASS VIALS**. For long-term storage, freeze reconstituted standards and controls at ≤ -20°C. Avoid multiple (>2) freeze/thaw cycles.
- **DO NOT FREEZE Antibody-Immobilized Beads or PE Antibodies**.

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8°C

REAGENTS SUPPLIED	CATALOG NUMBER	VOLUME	QUANTITY
MILLIPLEX® MAP Anti-Human κ and λ Light Chain, PE	HGAM-1301	50 µL	1 tube
MILLIPLEX® MAP Human IgE Standard	47-303	0.25 mL	1 vial
MILLIPLEX® MAP Human Serum Positive Control	PC-303E	0.25 mL	1 vial
MILLIPLEX® MAP Assay Buffer	L-AB	30 mL	1 bottle
MILLIPLEX® MAP Wash Buffer, 10X	L-WB	30mL	2 bottles
Set of one 96-Well Plates with 2 Sealers	---	---	1 plate 2 sealers

Human Immunoglobulin Isotyping Antibody-Immobilized Beads:

Bead/Analyte Name	Luminex Bead Region	Cat. #
Anti-Human IgE Bead	75	HIGE-MAG

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex Sheath Fluid (Luminex Catalogue #40-50000) or Luminex Drive Fluid (Luminex Catlogue # 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, Millipore catalog #40-015 or equivalent) or Hand held Magnetic Separation Block (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 Microtiter filter plate (MX-PLATE) to run the assay with the use of Vacuum Filtration Unit (Millipore Vacuum Manifold Catalog #MSVMHTS00, or equivalent. 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. On disposal, flush with a large volume of water to prevent azide build up.

TECHNICAL GUIDELINES

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

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.

- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- The Standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be transferred to polypropylene tubes and stored at ≤ -20°C for 1 month and at ≤ -80°C for greater than one month.
- If samples fall above the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes.
- 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 Luminex 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 disc.
- For cell culture supernatants, use the culture medium as the diluent in background, standard curve, and control wells. In assays using serum samples, all samples, standards, and controls should be diluted in Assay Buffer.
- 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 1:50 (4 µL sample diluted in 196 µL Assay Buffer) in the Assay Buffer and a standard curve diluted in Assay Buffer should be used.
- For data analysis, multiply the final concentration of each sample by the dilution factor.

B. 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.
- Dilute the sample to approximately 1 µg/mL Ig in Assay Buffer. [Cell culture supernatants samples approximately (1:5); bioreactor supernatants (1:100)]. Note: Cell culture supernatant concentrations are cell-line dependent and range from 5-50 µg/mL. Bioreactor supernatants may be as concentrated as 1 mg/mL.

NOTE:

- A maximum of 50 µL per well of diluted serum or supernatant 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 the antibody-bead vial for 30 seconds; vortex for 1 minute. Add 150 µL from the antibody bead vial to the Mixing Bottle and add 2.85 ml Assay Buffer to bring final volume to 3.0 mL. 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.)

B. Preparation of Standards

Resuspend MILLIPLEX MAP Human IgE Standard in 0.25 mL deionized water. Vortex at high speed for 15 seconds. Place on ice for 15 minutes. This is the 1000 ng/ml standard.

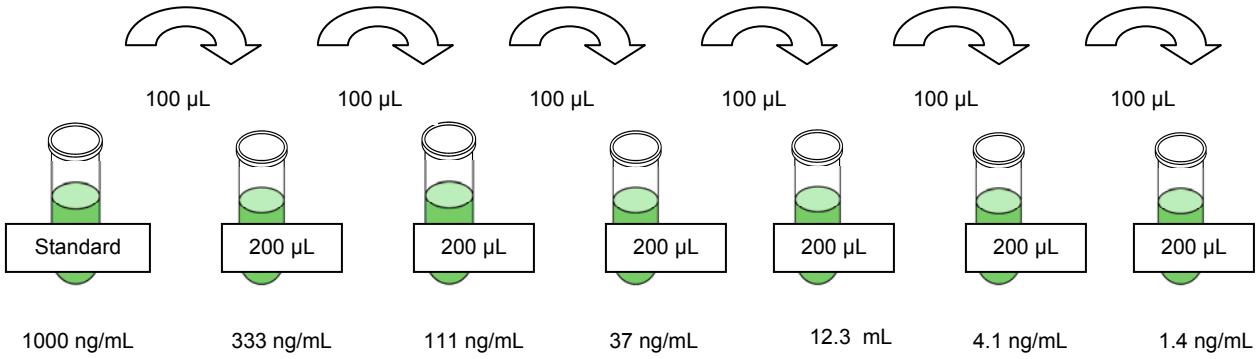
Preparation of Working Standards:

Label six polypropylene microfuge tubes as 330 ng/mL, 111 ng/mL, 37 ng/mL, 12 ng/mL, 4.1 ng/mL and 1.4 ng/mlL Add 200 µL of Assay Buffer to each of the six tubes. Prepare 3-fold serial dilutions by adding 100 µL of the reconstituted 1000 ng/mL to the 330 ng/mL tube, mix well and transfer 100 µL of the 330 ng/mL Std to the 111 ng/mL tube, mix well and transfer 100 µL of the 111 ng/mL Std to the 37 ng/mL tube, mix well and transfer 100 µL of the 37 ng/mL Std to the 12 ng/mL tube, mix well and transfer 100 µL of the 12 ng/mL Std to the 4.1 ng/mL tube and mix well and transfer 100 µL of the 4.1 ng/mL Std to the 1.4 ng/mL tube and mix well . The Standard 0 (Background) will be Assay Buffer.

Standard (ng/ml)	Volume of Water to Add	Volume of Standard to Add
1000	250 µL	0

Standard	Volume of Assay Buffer to Add	Volume of Standard to Add
333	200 µL	100 µL of 1000 ng/mL
111	200 µL	100 µL of 333 ng/mL
37	200 µL	100 µL of 111 ng/mL
12.3	200 µL	100 µL of 37 ng/mL
4.1	200 µL	100 µL of 12.3 ng/mL
1.4	200 µL	100 µL of 4.1 ng/mL

Preparation of Working Standards:



C. Preparation of Human Serum Positive Control

Rususpend MILLIPLEX MAP Human Serum Positive Control (Catalog # PC-303E) in 0.25 mL deionized water (or cell culture medium if running with cell culture supernatants). Vortex at high speed for 15 seconds. Place on ice for 15 minutes prior to use.

D. Preparation of Human κ and λ Light Chain, PE

To prepare 100X detection reagent, dilute anti-Human Kappa and Lambda-PE to working solution (1:100) with Assay Buffer (for a full plate, use 30 µL of the 100X anti-Human kappa-PE in 2.97 mL Assay Buffer.

E. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water. Store 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 Standards [0 (Background) though 1000 ng/ml], Positive Control, 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 50 µL Assay Buffer to each well of the Assay Plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Decant Assay Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 50 µL of control, standard, or diluted sample to appropriate wells. Use Assay Buffer for background wells.
4. Vortex the MILLIPLEX MAP Anti-Human IgE Bead at medium speed for 15 seconds, and then sonicate for 15 seconds using a sonication bath. Add 25 µL of bead solution to each well.
5. Cover with plate sealer and wrap with foil. Incubate 1 hour with agitation on plate shaker at room temperature.
6. Wash plate 2 times with 200 µL/well of Wash Buffer, removing Wash Buffer by aspiration between each wash. (NOTE: DO NOT INVERT PLATE unless using hand held magnetic separation block.) To avoid washing/aspiration related bead loss, allow 60 seconds between dispensing of the Wash Buffer and subsequent aspiration. If using the recommended plate washer for magnetic beads (Bio-Tek ELx405) follow the appropriate equipment settings outlined on Page 13. If using Hand Held Magnetic Separation Block (40-285), follow instructions included with the magnet.
7. Add 25 µL per well of diluted Anti-Human κ and λ light Chain, PE.
8. Cover with plate sealer and wrap with foil. Incubate 1 hour with agitation on plate shaker at room temperature.

Add 50 µL Assay Buffer per well

↓ Shake 10 min, RT

↓ Decant

- Add 50 µL Standard or Control to appropriate wells.
- Add 50 µL Assay Buffer to background wells.
- Add 50 µL diluted samples to sample wells.
- Add 25 µL Beads to each well.

↓ Incubate 1 hour at RT with shaking in the dark

↓ Wash plate 2X with 200 µL Wash Buffer

Add 25 µL κ and λ light chain PE solution per well

↓ Incubate 1 hour at RT with shaking in the dark

9. Remove fluid by aspiration. (NOTE: DO NOT INVERT PLATE unless using hand held magnetic separation block.) To avoid aspiration related bead loss, allow the plate to soak on the magnet of the plate washer for 60 seconds prior to aspiration.

10. Resuspend in 150 µL/well of Sheath Fluid (or Drive Fluid if using MAGPIX®) and proceed to reading results on an appropriate Luminex® instrument

Aspirate fluid from plate and add 150 µL Sheath Fluid or Drive Fluid per well. Read results using an appropriate Luminex® instrument.

QUALITY CONTROL

The ranges for the Human Serum Positive Control are provided on the card insert or can be located at the MILLIPORE website www.millipore.com/techlibrary/index.do using the catalog number as the keyword.

EQUIPMENT SETTINGS (Plate Reader)

These specifications are for the Luminex 200™ xPONENT™, FlexMAP 3D™, MAGPIX® and Luminex HTS. 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 (Millipore Cat.# 40-275) and performance verified by Verification Kit (Millipore Cat. # 40-276). The Luminex FlexMAP 3D™instrument must be calibrated with the FlexMAP 3D™ Calibration Kit (Millipore cat# 40-028) and performance verified with the FlexMAP 3D™ Performance Verification Kit (Millipore cat# 40-029). The Luminex MAGPIX® instrument must be calibrated with the MAGPIX® Calibration Kit (Millipore cat# 40-049 and performance verified with the MAGPIX® Performance Verification Kit (Millipore cat# 40-050).

NOTE: These assays cannot be run on Luminex 100™ instruments or any instruments using the 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.# 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
IgE Bead Setting	Bead Region # 75

EQUIPMENT SETTINGS (Plate Washer)

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

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. Bottom Wash first?: NO
6. 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.

Important - To avoid aspiration related bead loss, allow the plate to sit on the magnet of the plate washer for 60 seconds prior to starting the wash method.

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 manufacturers's recommendations for programming instructions.

ASSAY CHARACTERISTICS

Assay Sensitivities (minimum detectable concentrations, ng/mL)

MinDC: Minimum Detectable Concentration is calculated using Milliplex Analyst Software from Millipore. 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	*MinDC + 2SD*
IgE	**0.4**

Precision (%CV)

Intra-assay precision is generated from the mean of the %CV's from four reportable results across 1 concentration of analytes in one experiment. Inter-assay precision is generated from the mean of the %CV's from four reportable results across 1 concentration of analytes across 4 different assays.

Analyte	*Intra-Assay (%CV)*	*Inter-Assay (%CV)*
IgE	**<10**	**<13**

Accuracy (% Recovery)

Spike Recovery: The data represent mean percent recovery of 4 levels of spiked standards in diluted serum (1:50) from 4 different serum matrix.

Isotype	*Spike Recovery in Serum*
IgE	**102%**

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 disc.
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 light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.
Signal for whole plate is same as background	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Streptavidin-Phycoerythrin was not added	Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been removed, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been removed prior to adding Streptavidin Phycoerythrin	May need to repeat assay if desired sensitivity not achieved.
	Incubations done at inappropriate temperatures, timings or agitation	Assay conditions need to be checked.
Signals too high, standard curves are saturated	Calibration target value set too high	With some Luminex Instrument (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 0.2mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay setup 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 0.2mL 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 #**

Human IgE Standard	47-303
Human Serum Positive Control	PC-303E
Human κ and λ Light Chain, PE	HGAM-1301
10X Wash Buffer	L-WB
Assay Buffer	L-AB
Set of two 96-Well Plates with Sealers	MAG-PLATE

Antibody-Immobilized Magnetic Beads

Analytes	Bead #	Cat. #
Anti Human IgE	75	HIGE-MAG

ORDERING INFORMATION

To place an order:

To assure the clarity of your kit order, please FAX the following information to our customer service department:

- Your name, telephone and/or fax number
- Customer account number
- Shipping and billing address
- Purchase order number
- Catalog number and description of product
- Quantity of kits

FAX: (636) 441-8050
Toll-Free US: (800) MILLIPORE
Mail Orders: Millipore Corp.
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers in placing an order or obtaining additional information about MILLIPLEX MAP products, please contact your multiplex specialist or sales representative or email our European Customer Service at customerserviceEU@Millipore.com.

Conditions of Sale

All products are for research use only. They are not intended for use in clinical diagnosis or for administration to humans or animals. All products are intended for *in vitro* use only.

Material Safety Data Sheets (MSDS)

Material Safety Data Sheets for Millipore products may be ordered by fax or phone or through our website at www.millipore.com/techlibrary/index.do

WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 0 Background	37 ng/mL	Positive Control									
B	Standard 0 Background	37 ng/mL	Positive Control									
C	1.4 ng/mL	111 ng/mL										
D	1.4 ng/mL	111 ng/mL										
E	4.1 ng/mL	330 ng/mL										
F	4.1 ng/mL	330 ng/mL										
G	12 ng/mL	1000 ng/mL										
H	12 ng/mL	1000 ng/mL										