User Guide

# MILLIPLEX® Human Immunoglobulin IgE Single Plex Magnetic Bead Kit

## 96-Well Plate Assay

**HGAMMAG-303E**

# 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.

The MILLIPLEX® portfolio offers the broadest selection of analytes across a wide range of disease states and species. Once the analytes of interest have been identified, you can rely on the quality that we build into each kit to produce results you can trust. In addition to the assay characteristics listed in the protocol, other performance criteria evaluated during the verification process include: cross-reactivity, dilution linearity, kit stability, and sample behavior (for example, detectability and stability).

Each MILLIPLEX® panel and kit includes:

- Quality controls (QCs) provided to qualify assay performance
- Comparison of standard (calibrator) and QC lots to a reference lot to ensure lot-to-lot consistency
- Optimized serum matrix to mimic native analyte environment
- Detection antibody cocktails designed to yield consistent analyte profiles within panel

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

The MILLIPLEX® Human Immunoglobulin Isotyping Panel is part of the most versatile system available for Immune Response research. We partner with you to design, develop, analytically verify and build the most comprehensive library available for protein detection and quantitation. MILLIPLEX® products offer you 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 Immunoglobulin Isotyping Panel is to be used for the measurement of IgE in serum and tissue/cell lysate and culture supernatant samples.

**For research use only. Not for use in diagnostic procedures.**
**Please read entire protocol before use.**
**It is important to use same assay incubation conditions throughout your study.**

## Principle

MILLIPLEX® products are based on the Luminex® xMAP® technology - one of the fastest growing and most respected multiplex technologies offering applications throughout the life-sciences and capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded magnetic beads known as MagPlex®-C microspheres.

- Luminex® products use proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, distinctly colored bead sets of 500-5.6 µm polystyrene microspheres or 80-6.45 µm magnetic microspheres can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The following Luminex® instruments can be used to acquire and analyze data using two detection methods:
    - The Luminex® analyzers, Luminex® 200™, FLEXMAP 3D®, and xMAP® INTELLIFLEX, are flow cytometry-based instruments that integrate key xMAP® detection components, such as lasers, optics, advanced fluidics and high-speed digital signal processors.
    - The Luminex® analyzer (MAGPIX®), a CCD-based instrument that integrates key xMAP® capture and detection components with the speed and efficiency of magnetic beads.
- Each individual microsphere is identified, and the result of its bioassay is quantified based on fluorescent reporter signals. We combine the streamlined data acquisition power of Luminex® xPONENT® acquisition software with sophisticated analysis capabilities of the new MILLIPLEX® Analyst 5.1, integrating data acquisition and analysis seamlessly with all Luminex® instruments.

- xMAP® INTELLIFLEX runs on INTELLIFLEX software for instrument control, run setup and generating high quality data with flexible output options. Data can be exported in xPONENT® style CSV files for compatibility with many existing analytical applications, or in the new, customizable INTELLIFLEX file format. The INTELLIFLEX file format is intended for flexibility and simplicity, allowing the user to freely select which data points to include and to reduce the time to analysis.

The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP® technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.

## Storage Conditions Upon Receipt

- Recommended storage for kit components is 2-8 °C.
- For long-term storage, freeze reconstituted standards and controls at ≤ -20 °C. Avoid multiple (> 2) freeze/thaw cycles.
- **DO NOT FREEZE** Antibody-Immobilized Beads, Detection Antibody, and Streptavidin-Phycoerythrin.

## Reagents Supplied

Store all reagents at 2-8 °C

| Reagents | Volume | Quantity | Cat. No. |
|---|---|---|---|
| Anti-Human κ and λ Light Chain Detection Antibodies | 3.5 mL | 1 bottle | HGAM-1301-1 |
| Streptavidin-Phycoerythrin | 115 µL | 1 tube | 45-001D |
| Human IgE Standard | 0.25 mL | 1 vial | 47-303 |
| Human Serum Positive Control | 0.25 mL | 1 vial | PC-303E |
| Assay Buffer | 30 mL | 1 bottle | L-AB |
| Wash Buffer, 10X | 60 mL | 1 bottle | L-WB |
| Set of one 96-Well Plates with 2 Sealers | - | 1 set | - |

**Human Immunoglobulin Isotyping Antibody-Immobilized Beads**

| Bead/Analyte Name | Bead Region | Cat. No. |
|---|---|---|
| Anti-Human IgE Bead | 75 | HIGE-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 | |
|---|---|---|---|
| Human IgE Standard | 47-303 |   | **Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention. |
| 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. |
| Human Serum Positive Control | PC-303E |   | **Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention. |

# 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 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 needle probe is clean. This may be achieved by sonication and/or alcohol flushes.
- When reading the assay on the Luminex® 200™ instrument, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended filter plates using 3 alignment discs. When reading the assay on the MAGPIX® instrument, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate or to the recommended filter plates using 2 alignment discs. When reading the assay on the FLEXMAP 3D® instrument, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate using 1 alignment disc.
- For the FLEXMAP 3D® instrument, when using the solid plate in the kit, the final resuspension should be with 150 µL Sheath Fluid PLUS in each well and 75 µL should be aspirated.
- For 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 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

**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 1:50 (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.

**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

**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.)

**Preparation of Standards**

Resuspend MILLIPLEX® Human IgE Standard in 0.25 mL deionized water. Vortex at high speed for 15 seconds. Place on ice for 15 minutes. Transfer the reconstituted standard to a polypropylene microfuge tube and return to ice. This is the 1000 ng/ml standard.

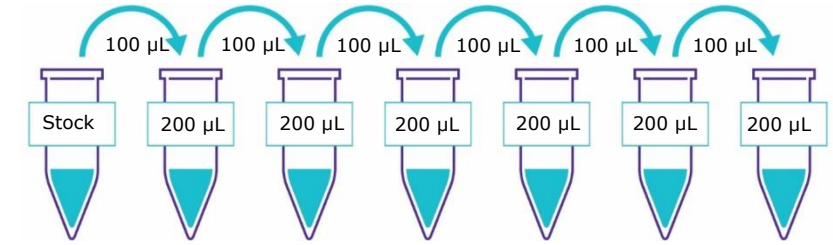
**Preparation of Working Standards:**

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/mL. 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) | Add Deionized Water (µL) | Add Standard (volume) |
|---|---|---|
| 1000 | 250 | 0 |

| Standard (ng/ml) | Add Assay Buffer (µL) | Add Standard (volume) |
|---|---|---|
| 333 | 200 | 100 µL of 1000 ng/mL |
| 111 | 200 | 100 µL of 333 ng/mL |
| 37 | 200 | 100 µL of 111 ng/mL |
| 12.3 | 200 | 100 µL of 37 ng/mL |
| 4.1 | 200 | 100 µL of 12.3 ng/mL |
| 1.4 | 200 | 100 µL of 4.1 ng/mL |

**Preparation of Standards**




| Reconstituted Standard 7 1000 ng/mL | Standard 6 333 ng/mL | Standard 5 111 ng/mL | Standard 4 37 ng/mL | Standard 3 12.3 ng/mL | Standard 2 4.1 ng/mL | Standard 1 1.4 ng/mL |
|---|---|---|---|---|---|---|

**Preparation of Human Serum Positive Control**

Resuspend MILLIPLEX® Human Serum Positive Control (Cat. No. 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. Transfer the reconstituted Positive Control into a polypropylene microfuge tube and return to ice.

**Preparation of Streptavidin-Phycoerythrin**

Dilute 75 µL of Streptavidin-Phycoerythrin (Cat. No. 45-001D) with 2.925 mL Assay Buffer. Vortex to mix.

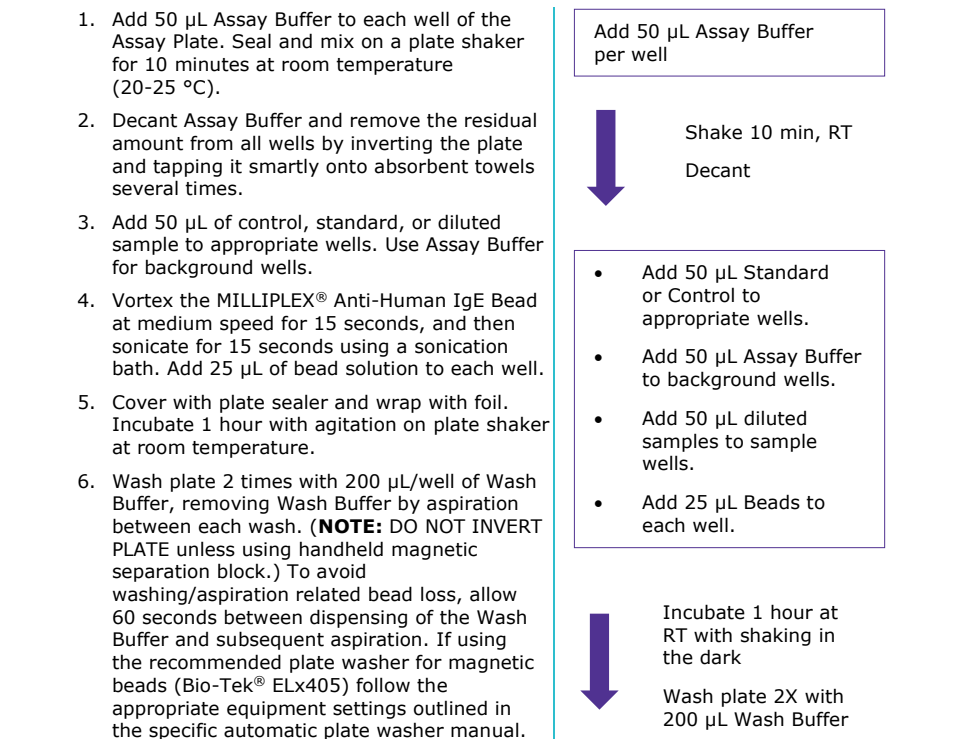
**Preparation of Wash Buffer**

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

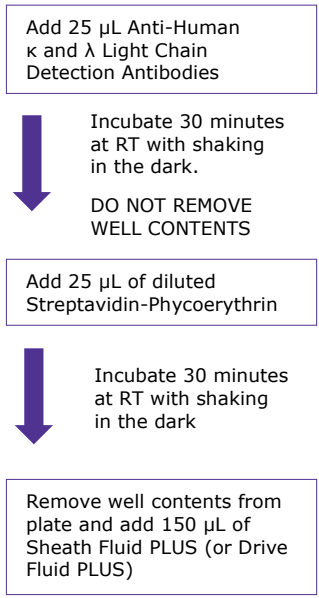
# Immunoassay Procedure

- 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® 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 handheld 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 in the specific automatic plate washer manual. If using Handheld Magnetic Separation Block (Cat. No. 40-285), follow instructions included with the magnet.



7. Add 25 µL of Anti-Human κ and λ Light Chain Detection Antibody.
8. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25 °C). **DO NOT REMOVE WELL CONTENTS AFTER INCUBATION.**
9. Add 25 µL of diluted Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibody.
10. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25 °C).
11. Remove fluid by aspiration. **DO NOT WASH**. (**NOTE:** DO NOT INVERT PLATE unless using handheld 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.
12. Resuspend in 150 µL/well of Sheath Fluid PLUS (or Drive Fluid PLUS if using MAGPIX®) and proceed to reading results on an appropriate Luminex® instrument.



# Plate Washing

**Solid Plate**

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

- Handheld magnet (Cat. No. 40-285)
  Rest plate on magnet for 60 seconds to allow complete settling of magnetic beads. Remove well contents by gently decanting the plate in an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid. Wash plate with 200 µL of Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps as recommended in Assay Procedure.

- Magnetic plate washer (Cat. No. 40-094, 40-095, 40-096 and 40-097)
  Please refer to specific automatic plate washer manual for appropriate equipment settings. Please note that after the final aspiration, there will be approximately 25 µL of residual wash buffer in each well. This is expected when using the BioTek® plate washer and this volume does not need to be aspirated from the plate.

If using an automatic plate washer other than BioTek® 405 LS or 405 TS, please refer to the manufacturer's recommendations for programming instructions.

**Filter Plate** (Cat. No. MX-PLATE)

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

# Equipment Settings

Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® 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 |
| IgE Bead Setting | Bead Region No. 75 |

# Quality Controls

The ranges for each analyte in the Positive Control are provided on the card insert or can be located at our website SigmaAldrich.com using the catalogue number as the keyword.

# Assay Characteristics

## Assay Sensitivities (minimum detectable concentrations, ng/mL)

Minimum Detectable Concentration (MinDC) is calculated using MILLIPLEX® Analyst 5.1. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions.

| Analyte | MinDC + 2SD |
|---|---|
| IgE | 0.4 ng/mL |

## 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

| 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. |
| | Matrix used has endogenous analyte or interference | Check matrix ingredients for cross reacting components (for example, interleukin modified tissue culture medium). |
| | Insufficient washes | Increase number of washes. |

| Problem | Probable Cause | Solution |
|---|---|---|
| Beads not in region or gate | Luminex® 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 (for example, Bio-Plex®) require different gate settings than those described in the kit protocol. Use the 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. |
| 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. |
| Signals too high, standard curves are saturated | Calibration target value set too high | With some Luminex® Instruments (for example, Bio-Plex®) default target setting for RP1 calibrator is set at high PMT. Use low target value for calibration and reanalyze plate. |
| | Plate incubation was too long with standard curve and samples | Use shorter incubation time. |

| Problem | Probable Cause | Solution |
|---|---|---|
| Sample readings are out of range | Samples contain no or below detectable levels of analyte | If below detectable levels, it may be possible to use higher sample volume. Check with technical 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 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 0.2mL 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 0.2 mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (blocked) plate and continue. |
| | Plate set directly on table or absorbent towels during incubations or reagent additions | Set plate on plate holder or raised edge so bottom of filter is not touching any surface. |
| | Insufficient blotting of filter plate bottom causing wicking | Blot the bottom of the filter plate well with absorbent towels after each wash step. |
| | Pipette touching plate filter during additions | Pipette to the side of plate. |
| | Probe height not adjusted correctly | Adjust probe to 3 alignment discs in well H6. |
| | Sample too viscous | May need to dilute sample. |

## Product Ordering

| Replacement Reagents | Cat. No. |
|---|---|
| Human IgE Standard | 47-303 |
| Human Serum Positive Control | PC-303E |
| Human κ and λ Light Chain Detection Antibodies | HGAM-1301-1 |
| Streptavidin-Phycoerythrin | 45-001D |
| 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 No. | Cat. No. |
|---|---|---|
| Anti Human IgE | 75 | HIGE-MAG |

## 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 | | | | | | | | | | |

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