User Guide

MILLIPLEX® Mouse Immunoglobulin Isotyping Magnetic Bead Panel

96-Well Plate Assay

MGAMMAG-300K

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. Immunoglobulin-deficiency disorders, such as autoimmune disease, some GI conditions and malignancies, are characterized by specific isotype deficiencies or varying concentrations of one or more isotypes. Disease states can range from the absence of one isotype class or subclass to a total deficiency of immunoglobulin classes. In addition, isotyping applications include analyzing hybridomas during antibody development.

We recognize the need to provide you with the ability to quantitate immunoglobulin classes and subclasses simultaneously. Our MILLIPLEX® Mouse Isotyping Kit has been designed to enable you to measure accurately mouse IgG subclasses (1, 2a, 2b, and 3), IgM, and IgA– all in one well. In addition, this configurable kit enables the customer to customize which subclasses are measured. The xMAP multiplex technology is ideal for measuring levels of these isotypes, which not only decreases the number of assays as well as the amount of sample required, but also greatly reduces the possible inaccuracies that result from performing multiple assays.

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® Mouse Isotyping Magnetic Bead Panel thus enables you to focus on the therapeutic potential of immunoglobulin Ig in immune response. 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® Mouse Isotyping Magnetic Bead Panel is part of the most versatile system available for immunology/immune response research. From our single to multiplex biomarker solutions, we partner with you to design, develop, analytically verify and build the most comprehensive library available for protein detection and quantitation.

MILLIPLEX® products offer you:

- The ability to choose any combination of analytes from our panel of 6 analytes to design a custom kit that better meets your needs.
- A convenient "all-in-one" box format that gives you the assurance that you will have all the necessary reagents you need to run your assay.

The MILLIPLEX Mouse Immunoglobulin Isotyping Magnetic Bead Panel is a 6-plex kit to be used for the simultaneous quantification of the following 6 analytes is mouse serum and tissue/cell lysate and culture supernatant samples: IgM, IgG1, IgG2a, IgG2b, IgG3 and IgA.

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.
- 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

Store all reagents at 2–8 °C

Reagents	Volume	Quantity	Cat. No.
MILLIPLEX® Anti-Mouse κ Light Chain, PE	50 µL	1 tube	44-029
MILLIPLEX® Anti-Mouse λ Light Chain, PE	26 µL	1 tube	44-030
MILLIPLEX® Mouse Multi-Immunoglobulin Standard	0.5 mL	1 vial	47-300
MILLIPLEX® Mouse Immunoglobulin Positive Control	0.25 mL	1 vial	43-008L
MILLIPLEX® Assay Buffer	30 mL	2 bottles	L-AB
MILLIPLEX® MAP Wash Buffer, 10X	60 mL	1 bottle	L-WB
Set of one 96-Well Plate with 2 Sealers	-	1 set	-

Mouse Immunoglobulin Isotyping Antibody-Immobilized Magnetic Beads

Bead/Analyte Name	Luminex® Bead Region	Customizable 6 Analytes (20X concentration, 200 µL) Available	Cat. No.
Anti-Mouse IgA Bead	18	✔	MIGA-MAG
Anti-Mouse IgG1 Bead	21	✔	MIGG1-MAG
Anti-Mouse IgG2a Bead	36	✔	MIGG2A-MAG
Anti-Mouse IgG2b Bead	51	✔	MIGG2B-MAG
Anti-Mouse IgG3 Bead	54	✔	MIGG3-MAG
Anti-Mouse IgM Bead	72	✔	MIGM-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. On disposal, flush with a large volume of water to prevent azide build up.

Symbol Definitions

Ingredient	Cat. No.	Label	
Mouse Immunoglobulin Positive Control	43-008L		**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.
Mouse Multi-Immunoglobulin Standard	47-300		**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.

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. 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 Luminex® 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 using 4 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. When reading the assay on the MAGPIX® instrument, adjust probe height according to the protocols recommended by Luminex® to the kit solid plate using 2 alignment discs.
- For xMAP® INTELLIFLEX, 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. In all cases, use Assay Buffer for resuspension steps.
- 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 1:25,000 in the Assay Buffer and a standard curve diluted in Assay Buffer should be used.
- To achieve a 1:25,000 dilution, dilute 5 µL of sample into 1245 µL of ultrapure water (1:250). Immediately dilute 5 µL of the 1:250 dilution into 495 µL of Assay Buffer (1:100) dilution.
- For data analysis, multiply the final concentration of each sample by the dilution factor.
- If prepared samples yield assay results outside of the standard curve, adjustment of the assay buffer dilution step may be necessary.

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 each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 150 µL from each antibody bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Assay Buffer. Vortex the mixed beads well. Unused portion may be stored at 2-8 °C for up to one month.
(**Note:** Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit.)

Example 1: When using 6 antibody-immobilized beads, add 150 µL from each of the 6 bead sets to the Mixing Bottle. Then add 2.1 mL Assay Buffer.

Preparation of Standards

Resuspend MILLIPLEX® Mouse Multi-Immunoglobulin (IgG1, IgG2a, IgG2b, IgG3, IgA, and IgM) Standard (Cat. No. 47-300) in 0.5 mL Assay Buffer (Cat. No. L-AB). Vortex at high speed for 15 seconds. Place on ice for 15 minutes. This is Standard 7. Transfer the reconstituted Standard into a polypropylene microfuge tube and return to ice.
Note: Standards are of kappa light chain isotype and therefore will react only with anti-Mouse Kappa-PE detection reagent.

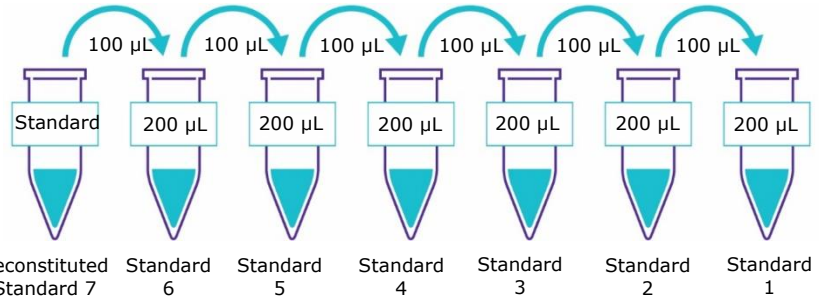
Preparation of Working Standards (Standard)

Label six polypropylene microfuge tubes Standard 6, Standard 5, Standard 4, Standard 3, Standard 2 and Standard 1. Add 200 µL of Assay Buffer to each of the six tubes. Prepare 3-fold serial dilutions by adding 100 µL of the reconstituted Standard 7 to the Standard 6 tube, mix well and transfer 100 µL of the Standard 6 to the Standard 5 tube, mix well and transfer 100 µL of the Standard 5 to the Standard 4 tube, mix well and transfer 100 µL of the Standard 4 to Standard 3 tube, mix well and transfer 100 µL of the Standard 3 to the Standard 2 tube, mix well and transfer 100 µL of the Standard 2 to the Standard 1 tube and mix well. The Standard 0 (Background) will be Assay Buffer.

Standard	Add Assay Buffer (µL)	Volume of Standard to Add
Original (Standard 7) (Refer to analysis sheet for exact concentration)	500	0

Standard	Add Assay Buffer (µL)	Volume of Standard to Add
Standard 6	200	100 µL of Standard 7
Standard 5	200	100 µL of Standard 6
Standard 4	200	100 µL of Standard 5
Standard 3	200	100 µL of Standard 4
Standard 2	200	100 µL of Standard 3
Standard 1	200	100 µL of Standard 2

Preparation of Working Standards



Preparation of Positive Control

Resuspend MILLIPLEX® Mouse Multi-Ig Positive Control (Cat. No. 43-008L) in 0.25 mL Assay Buffer (Cat. No. L-AB) (or cell culture medium if running with cell culture supernatants). Vortex at high speed for 15 seconds. Place on ice for 15 minutes. Transfer the reconstituted Positive Control into a polypropylene microfuge tube and return to ice.

Preparation of Detection Reagent

To prepare 100X detection reagent, dilute Anti-Mouse κ Light Chain, PE to working solution (1:100) with Assay Buffer (for a full plate, use 25 µL of the 100X Anti-Mouse kappa-PE in 2.475 mL assay buffer).
[**Note:** 95% of mouse antibodies have κ light chains, so in most instances, use of only the κ Light Chain Detection Reagent will be necessary. If determination of light chain is desired or samples show no signal with the κ light chain detection, vacuum plate and prepare anti-Mouse λ, PE the same as above. Repeat detection step with anti-mouse λ, PE and reread plate.]

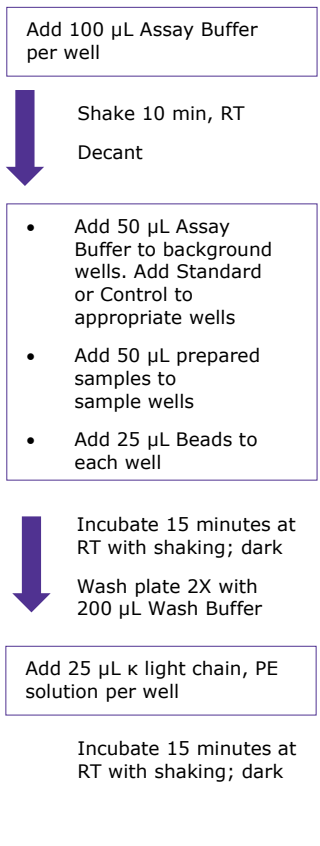
Preparation of Wash Buffer

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 7], 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, see special instruction on the next page.

1. Add 100 µL Assay Buffer to each well of the Assay Plate. Mix on a plate shaker for 10 minutes at room temperature.
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 Assay Buffer to background wells. Add 50 µL of standard, control or diluted sample to appropriate wells.
4. Vortex the MILLIPLEX® Anti-Mouse Multi-Immunoglobulin Beads 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 opaque plate cover and incubate 15 minutes with agitation on plate shaker at room temperature.
6. Wash plate 2 times with 200 µL/well of Wash Buffer, following instructions listed in the Plate Washing section.
7. Add 25 µL per well of prepared Anti-Mouse κ Light Chain, PE.
8. Cover with opaque plate cover and incubate 15 minutes with agitation on plate shaker at room temperature.
9. Gently remove contents following instructions listed in the Plate Washing section. **DO NOT WASH.** To avoid aspiration related bead loss, allow the plate to soak on the magnet plate for 60 seconds prior to aspiration or decanting.



10. Resuspend in 150 µL/well of Sheath Fluid PLUS (or Drive Fluid PLUS if using Magpix® instrument).
11. Proceed to reading results on an appropriate Luminex® instrument.
12. **OPTIONAL:** If no results are observed, or if determination of light chain is desired, aspirate plate and re-assay with Anti-Mouse λ Light Chain, PE.
13. Add 25 µL Anti-Mouse λ Light Chain, PE per well.
14. Cover with opaque plate cover and incubate 15 minutes with agitation on plate shaker at room temperature.
15. Remove fluid by aspiration and resuspend in 150 µL/well of Sheath Fluid PLUS (or Drive Fluid PLUS if using Magpix® instrument).
16. Proceed to reading results on an appropriate Luminex® instrument.

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

Continue only with samples where no results were observed

Aspirate Sheath Fluid PLUS

Add 25 µL λ light chain PE solution per well

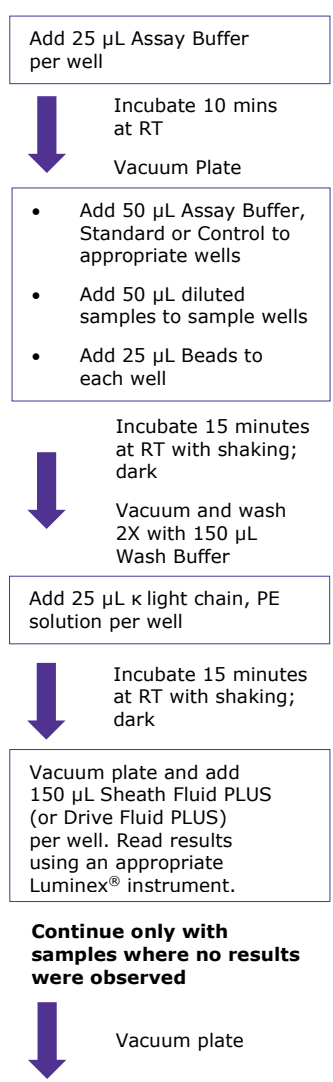
Incubate 15 minutes at RT with shaking; dark

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

Filter Plate Procedure

* 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. Pre-wet filter plate by pipetting 25 µL of Assay Buffer into each well. Seal and mix on a plate shaker for 10 minutes at room temperature. Remove Assay Buffer by vacuum. Blot bottom of plate with absorbent pad or paper towel.
2. Add 50 µL of Assay Buffer to background wells. Add 50 µL of standard, control or diluted sample to appropriate wells.
3. Vortex the MILLIPLEX® Anti-Mouse Multi-Immunoglobulin Beads 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.
4. Cover with opaque plate cover and incubate 15 minutes with agitation on plate shaker at room temperature.
5. Remove fluid by vacuum. Wash plate 2 times with 150 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Blot bottom of plate with absorbent pad or paper towel. **Do not over-dry.**
6. Add 25 µL per well of diluted Anti-Mouse κ Light Chain, PE.
7. Cover with opaque plate cover and incubate 15 minutes with agitation on plate shaker at room temperature.
8. Remove fluid by vacuum. Blot bottom of plate with absorbent pad or paper towel and resuspend in 150 µL/well of Sheath Fluid PLUS.
9. Proceed to reading results on an appropriate Luminex® instrument.
10. **OPTIONAL**: If no results are observed, or if determination of light chain is desired, vacuum plate and re-assay with Anti-Mouse λ Light Chain, PE.
11. Add 25 µL Anti-Mouse λ Light Chain, PE per well.



12. Cover with opaque plate cover and incubate 15 minutes with agitation on plate shaker at room temperature.
13. Remove fluid by vacuum and resuspend in 150 µL/well of Sheath Fluid PLUS (or Drive Fluid PLUS if using Magpix® instrument).
14. Proceed to reading results on an appropriate Luminex® instrument.

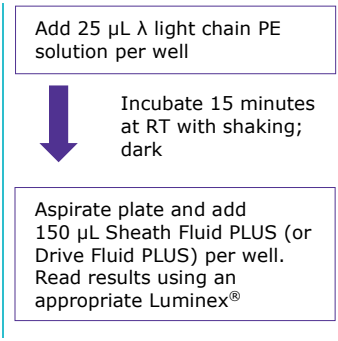


Plate Washing

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® with xPONENT® software and xMAP® INTELLIFLEX with INTELLIFLEX software:

These specifications are for the above listed instruments and software. Luminex® instruments with other software (for example, MasterPlex®, StarStation, LiquiChip, Bio-Plex® Manager™, LABScan™100) would need to follow instrument instructions for gate settings and additional specifications from the vendors for reading Luminex® magnetic beads.

For magnetic bead assays, each instrument must be calibrated and performance verified with the indicated calibration and verification kits.

Instrument	Calibration Kit	Verification Kit
Luminex® 200™ and HTS	xPONENT® 3.1 compatible Calibration Kit (Cat. No. LX2R-CAL-K25)	Performance Verification Kit (Cat. No. LX2R-PVER-K25)
FLEXMAP 3D®	FLEXMAP 3D® Calibrator Kit (Cat. No. F3D-CAL-K25)	FLEXMAP 3D® Performance Verification Kit (Cat. No. F3D-PVER-K25)
xMAP® INTELLIFLEX	xMAP® INTELLIFLEX Calibration Kit (Cat. No. IFX-CAL-K20)	xMAP® INTELLIFLEX Performance Verification Kit (Cat. No. IFX-PVER-K20)
MAGPIX®	MAGPIX® Calibration Kit (Cat. No. MPX-CAL-K25)	MAGPIX® Performance Verification Kit (Cat. No. MPX-PVER-K25)

NOTE: When setting up a Protocol using the xPONENT® software, you must select MagPlex® as the Bead Type in the Acquisition settings.

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

The Luminex® probe height must be adjusted to the plate provided in the kit. Please use Cat. No. MAG-PLATE, if additional plates are required for this purpose.

Events	50 per bead	
Sample Size	100 µL	
Gate Settings	8,000 to 15,000	
Reporter Gain	Default (Low PMT)	
Time Out	60 seconds	
Bead Set	Customizable 6-plex Bead No.	
	IgA	18
	IgG1	21
	IgG2a	36
	IgG2b	51
	IgG3	54
	IgM	72

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**
IgA	1.1
IgG1	0.27
IgG2a	0.67
IgG2b	0.18
IgG3	0.32
IgM	1.73

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 assay. 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)
IgA	10	17
IgG1	6	11
IgG2a	8	14
IgG2b	7	16
IgG3	5	13
IgM	5	14

Accuracy (% Recovery)

Spike Recovery: The data represent mean percent recovery of 4 levels of spiked standards in diluted serum from 4 different rat samples.

Isotype	Spike Recovery in Serum
IgA	98%
IgG1	96%
IgG2a	76%
IgG2b	98%
IgG3	85%
IgM	100%

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 incorrectly	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting 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, backflush 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 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 FLEXMAP 3D® instrument, 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 PLUS in each well and 75 µL should be aspirated. When reading the assay on 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 by pipetting with multichannel pipets 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® 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 °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 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 eliminate 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 incorrect 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® 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 tech support for appropriate protocol modifications.
	Samples contain analyte concentrations higher than highest standard point	Samples may require dilution and reanalysis for 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 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

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.

Product Ordering

Replacement Reagents	Cat. No.
MILLIPLEX® Anti-Mouse κ Light Chain, PE	44-029
MILLIPLEX® Anti-Mouse λ Light Chain, PE	44-030
MILLIPLEX® Mouse Multi-Immunoglobulin Standard	47-300
MILLIPLEX® Mouse Immunoglobulin Positive Control	43-008L
Wash Buffer	L-WB
Assay Buffer	L-AB
Set of two 96-Well Plates with 4 Sealers	MAG-PLATE

Antibody-Immobilized Magnetic Beads

Analytes	Bead No.	Cat. No.
Anti-Mouse IgA	18	MIGA-MAG
Anti-Mouse IgG1	21	MIGG1-MAG
Anti-Mouse IgG2a	36	MIGG2A-MAG
Anti-Mouse IgG2b	51	MIGG2B-MAG
Anti-Mouse IgG3	54	MIGG3-MAG
Anti-Mouse IgM	72	MIGM-MAG

Well Map

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 0 Background	Standard 4	Pos Control									
B	Standard 0 Background	Standard 4	Pos Control									
C	Standard 1	Standard 5										
D	Standard 1	Standard 5										
E	Standard 2	Standard 6										
F	Standard 2	Standard 6										
G	Standard 3	Standard 7										
H	Standard 3	Standard 7										

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Technical Assistance

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Safety Data Sheets (SDS)

Safety Data Sheets are available on the product page at SigmaAldrich.com.

MGAMMAG-300K Rev 10/21