User Guide

MILLIPLEX® Human Autoimmune Autoantibody Magnetic Bead Panel

96-Well Plate Assay

HAIAB-10K

Introduction

Autoantibody formation is principal to the pathogenesis of a variety of autoimmune diseases. Dysregulated apoptosis and the subsequent defective clearance of cellular debris leads to the exposure of autoantigens and the generation of autoantibodies. The presence of autoantibodies may indicate disease activity, prognosis, and clinical associations related to a variety of autoimmune diseases. These autoimmune diseases include, but are not limited to, Sjögren's Syndrome, Systemic Lupus Erythematosus, Mixed Connective Tissue Disease, Systemic Sclerosis, Calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactyly, telangiectasia (CREST syndrome), Polymyositis/Dermatomyositis, Vasculitis, Anti-Phospholipid Syndrome, and various overlap syndromes of these diseases.

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, kit stability, and sample behavior (for example, detectability and stability).

Each MILLIPLEX® panel meets stringent manufacturing criteria to ensure batch-to-batch reproducibility. The MILLIPLEX® Human Autoimmune Autoantibody Magnetic Bead Panel thus enables you to focus on the therapeutic potential of autoimmune autoantibodies. Coupled with the Luminex® xMAP® platform in a magnetic bead format, you receive the advantage of ideal speed and sensitivity, allowing multiplex detection of dozens of analytes simultaneously, which can dramatically improve productivity.

The MILLIPLEX® Human Autoimmune Autoantibody Magnetic Bead Panel is part of the most versatile system available for autoimmune autoantibody research. From our single to multiplex biomarker solutions, we partner with you to design, develop, analytically verify and build the most comprehensive library available for autoantibody detection.

MILLIPLEX® products offers you:

- The ability to choose any combination of analytes from our panel of 20 analytes to design a custom kit that better meets your needs.
- A convenient "all-in-one" box format that gives you the assurance that you will have all the necessary reagents you need to run your assay.

The MILLIPLEX® Human Autoimmune Autoantibody Magnetic Bead Panel is a 20-plex kit to be used for the simultaneous detection and profiling of any or all of the following analytes in serum and plasma samples: Anti-C1q, Anti-Centromere Protein A (CENP-A), Anti-Centromere Protein B (CENP-B), Anti-β 2-Glycoprotein, Anti-Jo-1, Anti-Ku, Anti-Mi-2, Anti-Myeloperoxidase, Anti-Proliferating cell nuclear antigen A (PCNA), Anti-Alanyl-tRNA synthetase (PL-12), Anti-PM/Scl-100, Anti-Proteinase 3, Anti-Ribosomal P, Anti-Ribonucleoprotein (RNP), Anti-RNP/Smith (RNP/Sm), Anti-Scl-70, Anti-Sm, Anti-Sjögren's Syndrome-related antigen B/La (SSB/La), Anti-Anti-Sjögren's Syndrome-related antigen A/Ro52 kDa (SSA/Ro52), Anti-Sjögren's Syndrome-related antigen A/Ro60 kDa (SSA/Ro60). For this particular panel, 4 assay control beads are included as part of the base kit format.

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

Principle

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

- Luminex® products use proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, distinctly colored bead sets of 500-5.6 µm polystyrene microspheres or 80-6.45 µm magnetic microspheres can be created, each of which is coated with a specific antigen.
- After an antibody from a test sample is captured by the bead, the PE-IgG conjugate is introduced to complete the reaction on the surface of each microsphere.
- The 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.
- **DO NOT FREEZE** Antigen-Immobilized Beads, Detection Antibody, and Streptavidin-Phycoerythrin.

Reagents Supplied

Store all reagents at 2-8 °C

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

* Contains 0.05% Proclin

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

Human Autoimmune Autoantibody Antigen-Immobilized Magnetic Beads

Bead/Antigen Name	Luminex® Magnetic Bead Region	Customizable 20 Antigens (50X concentration, 90 µL) Available	Cat. No.
SSA/Ro60 Beads-Magnetic	19	✔	HSSAR060-MAG
SSA/Ro52 Beads-Magnetic	21	✔	HSSAR052-MAG
SSB/La Beads-Magnetic	22	✔	HSSBLA-MAG
RNP Beads-Magnetic	25	✔	HRNP-MAG
RNP/Sm Beads-Magnetic	27	✔	HRNPSM-MAG
Sm Beads-Magnetic	29	✔	HSM-MAG
Ribosomal P Beads-Magnetic	34	✔	HRIBP-MAG
Proteinase 3 Beads-Magnetic	35	✔	HP3-MAG
Myeloperoxidase Beads-Magnetic	37	✔	HMYEP-MAG
β 2-Glycoprotein Beads-Magnetic	44	✔	HB2GLY-MAG
PCNA Beads-Magnetic	46	✔	HPCNA-MAG
CENP-A Beads-Magnetic	47	✔	HCENPA-MAG
CENP-B Beads-Magnetic	52	✔	HCENPB-MAG
Scl-70 Beads-Magnetic	53	✔	HSCL70-MAG
Jo-1 Beads-Magnetic	56	✔	HJ01-MAG
C1q Beads-Magnetic	57	✔	HC1Q-MAG
PM/Scl-100 Beads-Magnetic	61	✔	HPMSCL100-MAG
Ku Beads-Magnetic	63	✔	HKU-MAG
Mi-2 Beads-Magnetic	66	✔	HMI2-MAG
PL-12 Beads-Magnetic	74	✔	HPL12-MAG

Control - Antigen-Immobilized Magnetic Beads

Bead/Analyte Name	Luminex® Magnetic Bead Region	Control Beads (50X concentration, 90 µL) Available	Cat. No.
Control Bead 1-Magnetic	12	✔	CB1-MAG
Control Bead 2-Magnetic	13	✔	CB2-MAG
Control Bead 3-Magnetic	14	✔	CB3-MAG
Negative Control-Magnetic	15	✔	NCB-MAG

The 4 Control Beads are to be combined with the 20 Antigen-Immobilized Beads to make a 24-plex.

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 (VWR® Microplate Shaker Cat. No. 12620-926 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 Autoimmune Autoantibody Panel PE-IgG Conjugate	HAIAB-PEIGG		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
10X Wash Buffer–MILLIPLEX®	L-WB		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

Technical Guidelines

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

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

- For 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 serum/plasma samples that require further dilution beyond 1:100, use the Assay Buffer provided in the kit.
- Vortex all reagents well before adding to plate.

Sample Collection and Storage

Preparation of Serum Samples

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

Preparation of Plasma Samples

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

NOTE:

- A maximum of 25 µL per well of diluted serum or plasma can be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anti-coagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

Preparation of Reagents for Immunoassay

Preparation of Antigen-Immobilized Beads

For individual vials of beads, sonicate each antigen-bead vial for 30 seconds; vortex for 1 minute. Add 60 µL from each antigen or control bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Assay Buffer. Vortex the mixed beads well. Unused portion may be stored at 2-8 °C for up to one month.
(**Note:** Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit.)

Example 1: When using 10 antigen or control beads, add 60 µL from each of the 10 bead vials to the Mixing Bottle. Then add 2.4 mL Assay Buffer.

Example 2: When using 24 antigen or control beads, add 60 µL from each of the 24 bead vials to the Mixing Bottle. Then add 1.56 mL Assay Buffer.

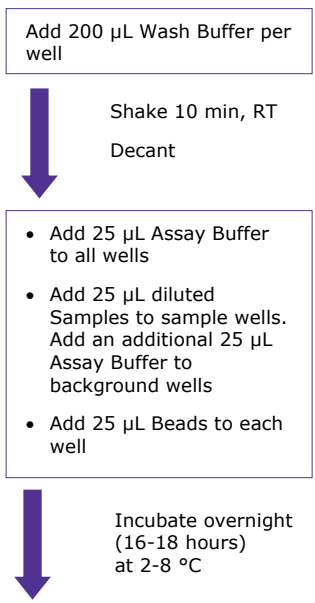
Preparation of Wash Buffer

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

Immunoassay Procedure

- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25 °C) before use in the assay.
- Diagram the placement of Background and Samples on Well Map Worksheet in a vertical configuration.
  (**Note:** Most instruments will only read the 96-well plate vertically by default.)
  It is recommended to run the assay in duplicate.
- If using a filter plate, set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

1. Add 200 µL of Wash Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25 °C).
2. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 25 µL of Assay Buffer to the all wells.
4. Add 25 µL of Sample (diluted) into the appropriate wells. Add an additional 25 µL Assay Buffer to background wells.
5. Vortex Mixing Bottle and add 25 µL of the Mixed Beads to each well.
   (**Note:** During addition of Beads, shake bead bottle intermittently to avoid settling.)
6. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker overnight (16-18 hours) at 2-8 °C.



7. Gently remove well contents and wash plate 3 times following instructions listed in the Plate Washing section.

8. Add 50 µL of Human Autoimmune Autoantibody Panel PE-IgG Conjugate into each well.
(**Note:** Allow the PE-IgG Conjugate to warm to room temperature prior to addition.)

9. Seal, cover with foil and incubate with agitation on a plate shaker for 90 minutes at room temperature (20-25 °C).

10. Gently remove well contents and wash plate 3 times following instructions listed in the Plate Washing section.

11. Add 150 µL of Sheath Fluid PLUS (or Drive Fluid PLUS if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.

12. Run plate on Luminex® 200™, HTS, FLEXMAP 3D®, MAGPIX® instrument with xPONENT® software or xMAP® INTELLIFLEX instrument with INTELLIFLEX software.

13. Save and analyze the Median Fluorescent Intensity (MFI) data. A working cut-off for a positive result can be established using MFI data from known negative sample population.

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 50 µL PE-IgG Conjugate per well

Incubate 90 minutes at RT

Remove well contents and wash 3X with 200 µL Wash Buffer

Add 150 µL Sheath Fluid PLUS or Drive Fluid PLUS per well

Read on Luminex® instrument (100 µL, 50 beads per bead set)

Plate Washing

Solid Plate

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

- Handheld magnet (Cat. No. 40-285)
  Rest plate on magnet for 60 seconds to allow complete settling of magnetic beads. Remove well contents by gently decanting the plate in an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid. Wash plate with 200 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	
Bead Set	Customizable 24-plex Beads	
	Control Bead 1	12
	Control Bead 2	13
	Control Bead 3	14
	Negative Control Beads	15
	SSA/Ro60 Beads	19
	SSA/Ro52 Beads	21
	SSB/La Beads	22
	RNP Beads	25
	RNP/SM Beads	27
	Sm Beads	29
	Ribosomal P Beads	34
	Proteinase 3 Beads	35
	Myeloperoxidase Beads	37
	β 2-Glycoprotein Beads	44
	PCNA Beads	46
	CENP-A Beads	47
	CENP-B Beads	52
	Scl-70 Beads	53
	Jo-1 Beads	56
	C1q Beads	57
	PM/Scl-100 Beads	61
	Ku Beads	63
	Mi-2 Beads	66
	PL-12 Beads	74

Assay Characteristics

Precision

Intra-assay precision is < 15% for this assay generated from the mean of the %CV's from 8 reportable results in a single assay. Inter-assay precision is < 20% for this assay generated from the mean of the %CV's across 4 different assays.

Troubleshooting

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. 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®.

Problem	Probable Cause	Solution
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.
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 instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex® instrument 4 times to rid it of air bubbles, wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.
	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.

Problem	Probable Cause	Solution
Signal for whole plate is same as background	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.
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.
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 0.2 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.
Human Autoimmune Autoantibody Panel PE-IgG Conjugate	HAIAB-PEIGG
Assay Buffer	L-AB
Set of two 96-Well plates with sealers	MAG-PLATE
10X Wash Buffer	L-WB
SSA/Ro60 Beads-Magnetic	HSSARO60-MAG
SSA/Ro52 Beads-Magnetic	HSSAR052-MAG
SSB/La Beads-Magnetic	HSSBLA-MAG
RNP Beads-Magnetic	HRNP-MAG
RNP/Sm Beads-Magnetic	HRNPSM-MAG
Sm Beads-Magnetic	HSM-MAG
Ribosomal P Beads-Magnetic	HRIBP-MAG
Proteinase 3 Beads-Magnetic	HP3-MAG
Myeloperoxidase Beads-Magnetic	HMYEP-MAG
β 2-Glycoprotein Beads-Magnetic	HB2GLY-MAG
PCNA Beads-Magnetic	HPCNA-MAG
CENP-A Beads-Magnetic	HCENPA-MAG
CENP-B Beads-Magnetic	HCENPB-MAG
Scl-70 Beads-Magnetic	HSCL70-MAG
Jo-1 Beads-Magnetic	HJ01-MAG
C1q Beads-Magnetic	HC1Q-MAG
PM/Scl-100 Beads-Magnetic	HPMSCL100-MAG
Ku Beads-Magnetic	HKU-MAG
Mi-2 Beads-Magnetic	HMI2-MAG
PL-12 Beads-Magnetic	HPL12-MAG
Control Bead 1-Magnetic	CB1-MAG
Control Bead 2-Magnetic	CB2-MAG
Control Bead 3-Magnetic	CB3-MAG
Negative Control-Magnetic	NCB-MAG

Well Map

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

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