Mouse IGF Binding Protein (IGFBP) Magnetic Bead Panel

96-Well Plate Assay

Cat. # MIGFBPMAG-43K

MILLIPLEX® MAP

MOUSE IGF BINDING PROTEIN (IGFBP) MAGNETIC BEAD PANEL KIT
96 Well Plate Assay

MIGFBPMAG-43K

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

INTRODUCTION

Insulin-like growth factor (IGF) binding proteins (IGFBP) are expressed by multiple tissues and cells, including hepatic cells. By binding both IGF-1 and IGF-2, IGFBPs play a critical role in the regulation of IGF action, altering IGF interaction with cell surface receptors and increasing circulating IGF half-life and bioavailability. While their functions at the cellular level are not fully understood, studies have demonstrated the capacity to both stimulate and inhibit IGF-1 activity, depending on the system involved and the IGFBP studied. The ability of IGFBPs to regulate cell sensitivity by inhibiting IGF signaling may function in the control of tumor cell growth.

MILLIPLEX® MAP offers the most versatile system available for IGFBP research to enable you to understand better the complexities of IGF signaling and the pathobiology of associated disease. Magnetic Beads can make the process of automation and high throughput screening easier with features such as walk-away washing. Advantages of Magnetic Beads include:

- More flexible plate and plate washer options
- Improved performance with turbid serum/plasma samples
- Assay results equivalent to non-magnetic beads
- Automated washing eliminates technical obstacles (i.e. clogging of wells) which may result during vacuum manifold washing.

Millipore's MILLIPLEX® MAP Mouse IGFBP Panel Kit is the most versatile system available for Insulin-Like Growth Factor Binding Protein (IGFBP) research.

- MILLIPLEX® MAP offers you the ability to:
    - Simultaneously quantify the concentration of six different IGFBPs.
    - 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 gives you the assurance that you will have all the necessary reagents you need to run your assay.

Millipore's MILLIPLEX® MAP Mouse Insulin-like Growth Factor Binding Protein (IGFBP) Multiplex Panel is to be used for the simultaneous quantification of IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-5, IGFBP-6, and IGFBP-7. This kit may be used for the analysis of all or any combination of the above analytes in diluted mouse serum and plasma samples, and cell culture conditioned-media samples.

This kit is for research purposes only.

Please read entire protocol before use.

It is important to use same assay incubation conditions throughout your study.

PRINCIPLE

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

- Luminex® uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, 100 distinctly colored bead sets can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin PE-conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The microspheres are allowed to pass rapidly through a laser which excites the internal dyes marking the microsphere set. A second laser excites PE, the fluorescent dye on the reporter molecule.
- Finally, high-speed digital-signal processors identify each individual microsphere and quantify the result of its bioassay based on fluorescent reporter signals.

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

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- Once the standards and controls have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE RECONSITUTED 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, Detection Antibodies, and Streptavidin-Phycoerythrin**.

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8 °C

REAGENTS SUPPLIED	CATALOG NUMBER	VOLUME	QUANTITY
Mouse IGFBP Standard	MIGFBP-8043	lyophilized	1 vial
Mouse IGFBP Quality Controls 1 and 2	MIGFBP-6043	lyophilized	2 vials
Serum Matrix Note: Contains 0.08% Sodium Azide	MIGF-SM	lyophilized	1 vial
Set of one 96-Well Black Plate with 2 Sealers	----------	----------	1 plate 2 sealers
Assay Buffer	LE-ABGLP	30 mL	2 bottles
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	2 bottles
Mouse IGFBP Detection Antibodies	MIGFBP-1043	5.5 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE	5.5 mL	1 bottle
Mixing Bottle	----------	----------	1 bottle

Mouse IGFBP Antibody-Immobilized Magnetic Beads:

Bead/Analyte Name	Luminex Bead Region	Customizable 6 Analytes (20X concentration, 200 µL) Available	Cat. #
Anti-IGFBP-1 Bead	27	✓	MIGFBP1-MAG
Anti-IGFBP-2 Bead	39	✓	MIGFBP2-MAG
Anti-IGFBP-3 Bead	42	✓	MIGFBP3-MAG
Anti-IGFBP-5 Bead	55	✓	MIGFBP5-MAG
Anti-IGFBP-6 Bead	61	✓	MIGFBP6-MAG
Anti-IGFBP-7 Bead	72	✓	MIGFBP7-MAG

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex Sheath Fluid (Luminex Corporation, catalog #40-50000) or Luminex Drive Fluid (Luminex Catalogue # MPXDF-4PK)

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 5 µL to 1000 µL
2. Multichannel Pipettes capable of delivering 25 µL to 200 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Aluminum Foil
7. Absorbent Pads
8. Laboratory Vortex Mixer
9. Sonicator (Branson Ultrasonic Cleaner Model #B200, or equivalent)
10. Titer Plate Shaker (Labline Scientific Instruments, Model #4625, or equivalent)
11. Luminex 200™, HTS, FLEXMAP 3D™, or MAGPIX® with xPONENT software by Luminex Corporation
12. Automatic Plate washer for magnetic beads (Bio-Tek ELx405, Millipore catalog #40-015 or equivalent) or Hand held Magnetic Separation Block (Millipore catalog # 40-285 or equivalent)

Note: If a plate washer or hand held magnetic separation block for magnetic beads is not available, one can use a microtiter filter plate (Millipore Catalog #MX-PLATE) to run the assay using a Vacuum Filtration Unit (Millipore Vacuum Manifold Catalog #MSVMHTS00 or equivalent with Millipore Vacuum Pump Catalog #WP6111560 or equivalent).

SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, sodium azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

TECHNICAL GUIDELINES

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

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.

TECHNICAL GUIDELINES (Continued)

- 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 an 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.
- After hydration, all Standards and Controls must be transferred to polypropylene tubes.
- The Standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock ("Standard 7") which may be stored at ≤ -20°C for 1 month and at ≤ -80°C for greater than one month.
- If sample values fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- Any unused, mixed Antibody-Immobilized Beads may be stored in the Bead Mixing Bottle at 2-8°C for up to one month.
- During the preparation of the standard curve, be certain to mix the higher concentration well before making the next dilution. In addition, use a new tip for 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 of the IGF Binding Proteins.
- The titer plate shaker should be set at a speed that provides 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.
- Prior to reading the plate, ensure that the needle probe of the Luminex® apparatus is not clogged. This may be achieved by sonication and/or alcohol flushes.
- When reading the assay on Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 4 alignment discs. When reading the assay on FLEXMAP 3D™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 1 alignment disc. When reading the assay on MAGPIX, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 2 alignment disc.
- For cell culture supernatants or cell/tissue extracts, use the culture or extraction medium as the matrix solution for blank, standard, and quality control wells.
- For serum and plasma samples, use the Serum Matrix Solution provided in this kit, as the matrix solution for blank, standard, and quality control wells.
- For cell/tissue homogenates, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Centrifuge samples before use in the assay to avoid insoluble debris and lipids.
- Vortex all reagents well before adding to plate.

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- To prevent possible IGFBP proteolysis, it is recommended that a protease inhibitor cocktail solution be added to each blood sample after blood collection.
- Allow the blood to clot for 30 minutes at room temperature. Then, centrifuge the samples for 10 minutes at 1000 x g. Finally, collect the serum samples and use them immediately in the assay or aliquot and store them at ≤ -20ºC.
- Avoid multiple (>2) freeze/thaw cycles.
- Prior to use, it is recommended to mix samples well by vortexing and then centrifuge them at 10,000 x g for 3-5 minutes. Centrifugation will ensure avoidance of insoluble debris and/or lipids.
- After centrifugation, serum samples should be diluted 25-fold in the supplied Assay Buffer. After the assay, calculated sample values should be multiplied by 25 to determine the actual concentration of each IGFBP within the undiluted samples. Some samples may need more or less dilution for IGFBP3 or IGFBP5 determination, respectively.

B. Preparation of Plasma Samples:

- To prevent possible IGFBP proteolysis, it is recommended that a protease inhibitor cocktail solution be added to each blood sample after blood collection.
- Blood collection using EDTA as an anti-coagulant is recommended for the collection of plasma samples. In addition, immediately after collection, the blood samples should be placed on ice and centrifuged at 4 degrees C for 10 minutes at 1000x g. Finally, collect the plasma samples and use them immediately in the assay or aliquot and store them at ≤ -20ºC.
- Avoid multiple (>2) freeze/thaw cycles.
- Prior to use, it is recommended to mix samples well by vortexing and then centrifuge them at 10,000 x g for 3-5 minutes. Centrifugation will ensure avoidance of insoluble debris and/or lipids.
- After centrifugation, plasma samples should be diluted 25-fold in the supplied Assay Buffer. After the assay, calculated sample values should be multiplied by 25 to determine the actual concentration of each IGFBP within the undiluted samples. Some samples may need more or less dilution for IGFBP3 or IGFBP5 determination, respectively.

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the samples at 10,000 x g for 3-5 minutes to remove insoluble debris and use them immediately for the assay or aliquot and store them at ≤ -20ºC.
- Avoid multiple (>2) freeze/thaw cycles.
- While undiluted tissue culture supernatants have been successfully used with this assay, some culture conditions may require dilution of samples in the appropriate culture medium.

NOTE:

- A maximum of 25 µL per well of 25-fold diluted serum or plasma sample, or 25 µL per well of neat cell culture, conditioned-media sample should 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 collected blood.

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

Sonicate each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 150 µL of each antibody-bead to the Mixing Bottle and bring the final volume to 3.0 mL with Assay Buffer. Vortex the mixture of antibody-immobilized beads well, prior to addition to the wells. Unused portions 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 2 antibody-immobilized beads, add 150 µL from each of the five bead sets to the mixing bottle. Then add 2.7 mL of Assay Buffer.

Example 2: when using 6 antibody-immobilized beads, add 150 µL from each of the seven bead sets to the mixing bottle. Then add 2.1 mL of Assay Buffer.

B. Preparation of Quality Controls

Before use, reconstitute both Quality Control 1 and Quality Control 2 with 250 µL of deionized water. Invert the vials several times to mix and vortex. Allow the vials to sit for 5-10 minutes and then transfer the controls to appropriately labeled polypropylene microfuge tubes. Unused portions may be stored at ≤ -20°C for up to one month.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water. Store unused portions at 2-8°C for up to one month.

D. Preparation of Serum Matrix

This step is required for serum or plasma samples only.

Add 1.0 mL deionized water to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 10 minutes for complete reconstitution. Left-over reconstituted Serum Matrix can be stored at ≤ -20°C for up to one month.

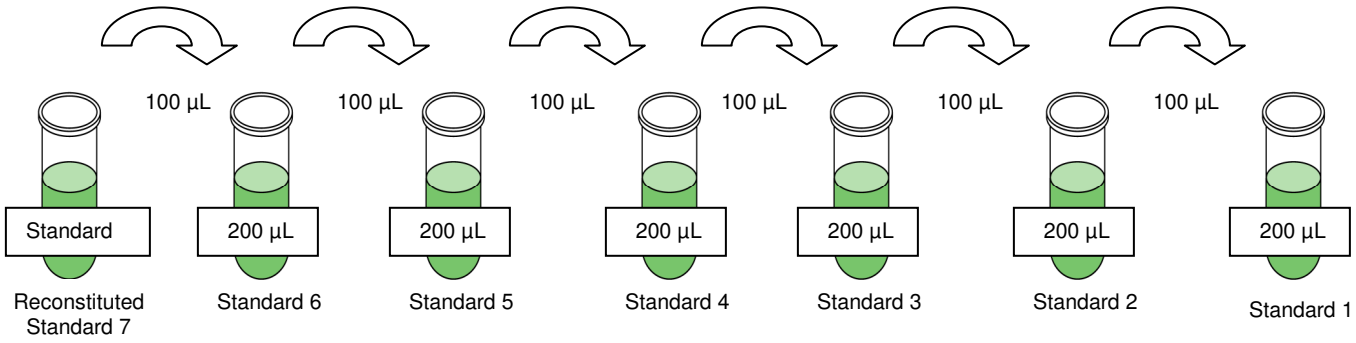
E. Preparation of Mouse IGFBP Standard

1. Prior to use, reconstitute the Mouse IGFBP Standard with 250 µL deionized water. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes, vortex and then transfer the standard to an appropriately labeled polypropylene microfuge tube. This will be used as Standard 7

2. Preparation of Working Standards

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. Perform 3 times serial dilutions by adding 100 µL of the “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 the “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 “Standard 1” well. The “Standard 0” (Background Control) will be Assay Buffer.

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



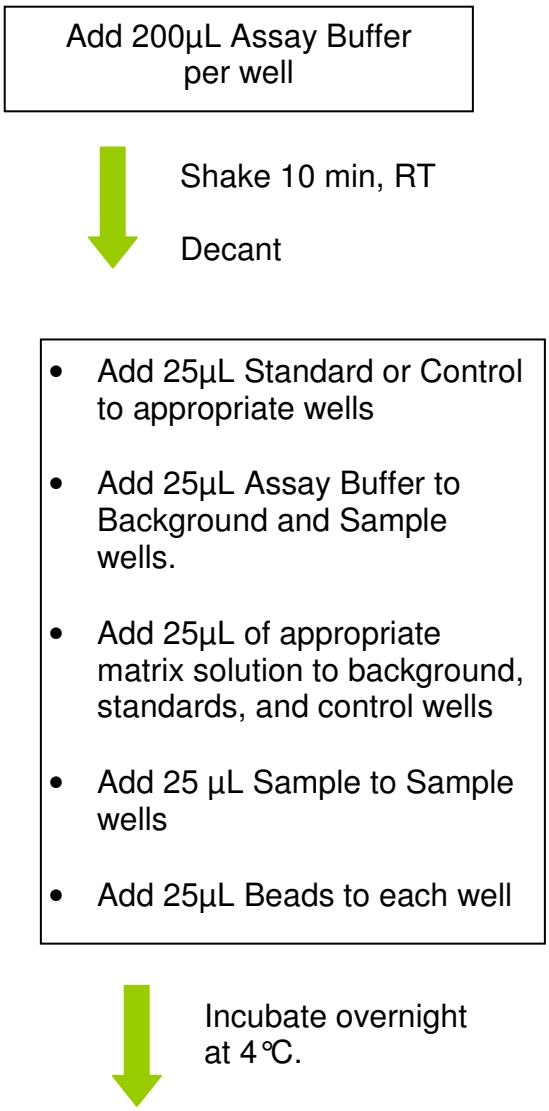
After serial dilution, the tubes should have the following concentrations for constructing standard curves.

Standard tube	IGFBP-1 (ng/mL)	IGFBP-2 (ng/mL)	IGFBP-3 (ng/mL)	IGFBP-5 (ng/mL)	IGFBP-6 (ng/mL)	IGFBP-7 (ng/mL)
Standard 1	0.04	0.04	0.04	0.41	0.04	0.04
Standard 2	0.12	0.12	0.12	1.23	0.12	0.12
Standard 3	0.37	0.37	0.37	3.70	0.37	0.37
Standard 4	1.11	1.11	1.11	11.11	1.11	1.11
Standard 5	3.33	3.33	3.33	33.33	3.33	3.33
Standard 6	10.00	10.00	10.00	100.00	10.00	10.00
Standard 7	30.00	30.00	30.00	300.00	30.00	30.00

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), 1, 2, 3, 4, 5, 6, 7], Controls 1 and 2, 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 Assay 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 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 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for "Standard 0" (Background Control).
4. Add 25 µL of Assay Buffer to the Sample wells.
5. Add 25 µL of appropriate matrix solution to the Background, Standards, and Control wells. When assaying serum or plasma, use the Serum Matrix provided in the kit. When assaying Cell Culture or other supernatant, use proper control culture medium as the matrix solution.
6. Add 25 µL of Sample into the appropriate wells.
7. Vortex Mixed Beads and add 25 µL of the Mixed Beads to each well. (Note: during addition of Mixed Beads, shake the bead mix intermittently to avoid settling.)
8. Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker for overnight (16-18 hours) incubation at 4°C .



9. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
10. Add 50 µL of Detection Antibodies into each well. (Note: allow the Detection Antibodies to warm to room temperature prior to addition.)
11. Seal, cover with foil, and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C)

    NOTE: DO NOT ASPIRATE AFTER INCUBATION
12. Add 50 µL Streptavidin-Phycoerythrin to each well containing the 50 µL of Detection Antibodies.
13. Seal, cover with foil, and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
14. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section..

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

Add 50µL Detection Antibody per well

Incubate for 30 minutes at RT

Do Not Aspirate

Add 50 µL Streptavidin-Phycoerythrin per well

Incubate for 30 minutes at RT

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

15. Add 100 µL of Sheath Fluid (or Drive Fluid if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.
16. Run plate on Luminex 200™ IS, HTS, FLEXMAP 3D™ or MAGPIX® with xPONENT software.
17. Save and analyze the median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method. Then, calculate the concentration of each IGFBP in the samples. (Note: For diluted samples, multiply the calculated concentration by the dilution factor.)

Add 100µL Sheath Fluid or Drive Fluid per well

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

PLATE WASHING

1.) Solid Plate
If using a solid plate, use either a hand-held magnet or magnetic plate washer.

A.) For hand-held magnet, 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 uL of Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps as recommended in Assay Procedure.

B.) For magnetic plate washer, let plate "soak" on magnet for 60 seconds to allow complete settling of the magnetic beads. Remove well contents by aspiration. Wash plate with 200 µL/well of Wash Buffer, letting beads "soak" for 60 seconds and removing Wash Buffer by aspiration after each wash. Repeat wash steps as recommended in Assay Procedure. **Note:** If using the recommended plate washer for magnetic beads (Bio-Tek ELx405) follow the appropriate equipment settings outlined in **EQUIPMENT SETTINGS.**

2.) Filter Plate (Millipore Cat# MX-PLATE)
If using a filter plate, use a vacuum filtration manifold to remove well contents. Wash plate 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

Bio-Tek ELx405:

The general recommended wash protocol (Link Protocol) is as follows:

Soak Program:	Wash Program:
Soak →	Aspirate→Dispense→Soak→Aspirate→Dispense→Soak→Aspirate →Dispense→Soak→Aspirate

1.) Soak program:
1. Soak duration: 60 sec
2. Shake before soak?: NO

2.) Wash program:

Method:
1. Number of cycles: 3
2. soak/shake: YES
3. Soak duration: 60 sec
4. Shake before soak: NO
5. Prime after soak: NO

Dispense:

1. Dispense volume: 200 µL/well
2. Dispense flow rate: 5
3. Dispense height: 130 (16.51 mm)
4. Horizontal disp pos: 00 (0 mm)
5. Bottom Wash first?: NO
6. Prime before start?: NO

Aspiration:

1. Aspirate height: 35 (4.445 mm)
2. Horizontal Asp Pos: 30 (1.372 mm)
3. Aspiration rate: 06 (15.0 mm/sec)
4. Aspiration delay: 0
5. Crosswise Aspir: NO
6. Final Aspir: YES
7. Final Aspir delay: 0 (0 msec)

3.) Link program: (**Note:** this is the program to use during actual plate washing). Link together the Soak and Wash programs outlined above.

Note: After the final aspiration, there will be approximately 25 µl of residual Wash Buffer in each well. This is expected when using the BioTek Plate washer and this volume does not need to be aspirated from the plate.

If using an automatic plate washer other than BioTek ELx405, please refer to the manufacturer's recommendations for programming instructions.

Luminex 200™, HTS, FLEXMAP 3D™ and MAGPIX® with xPONENT software:

These specifications are for the Luminex 200™, Luminex HTS, Luminex FLEXMAP 3D™ and Luminex MAGPIX® with xPonent software. Luminex instruments with other software (e.g. MasterPlex, StarStation, LiquiChip, Bio-Plex, LABScan100) would need to follow instrument instructions for gate settings and additional specifications from the vendors for reading Luminex Magnetic Beads.

For magnetic bead assays, the Luminex 200™ and HTS instruments must be calibrated with the xPonent 3.1 compatible Calibration Kit (Millipore Cat.# 40-275) and performance verified with the Performance Verification Kit (Millipore Cat. # 40-276). The Luminex FLEXMap 3D™ instrument must be calibrated with the FLEXMAP 3D™ Calibrator Kit (Millipore cat#40-028) and performance verified with the FLEXMAP 3D™ Performance Verification Kit (Millipore cat#40-029). The Luminex MAGPIX® instrument must be calibrated with the MAGPIX® Calibration Kit (Millipore cat# 40-049 and performance verified with the MAGPIX® Performance Verification Kit (Millipore cat# 40-050).

NOTE: These assays cannot be run on Luminex 100™ instruments or any instruments using Luminex IS 2.3 or Luminex 1.7 software.

The Luminex probe height must be adjusted to the plate provided in the kit. Please use Cat.# MAG-PLATE, if additional plates are required for this purpose.

Events:	50, per bead	
Sample Size:	50 µL	
Gate Settings	8,000 to 15,000	
Time Out	60 seconds	
Bead Set:	6-Plex Beads	
	IGFBP-1	27
	IGFBP-2	39
	IGFBP-3	42
	IGFBP-5	55
	IGFBP-6	61
	IGFBP-7	72

QUALITY CONTROLS

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at the Millipore website www.millipore.com/techlibrary/index.do using the catalog number as the keyword.

ASSAY CHARACTERISTICS

Cross-Reactivity

There was no or negligible (<2%) cross-reactivity between the antibodies and any of the other analytes in this panel.

Assay Sensitivities (minimum detectable concentrations, ng/mL)

MinDC: Minimum Detectable Concentration is given in ng/mL and is calculated by the StatLIA® Immunoassay Analysis Software from Brendan Technologies. 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	Min DC + 2SD
IGFBP-1	0.037
IGFBP-2	0.018
IGFBP-3	0.039
IGFBP-5	0.321
IGFBP-6	0.063
IGFBP-7	0.025

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentrations of IGFBP in a single assay. Inter-assay precision is generated from the mean of the %CV's from two different concentrations of IGFBP across 6 different assays.

Analyte	Intra-assay %CV	Inter-assay %CV
IGFBP-1	1.7%	6.9%
IGFBP-2	1.6%	6.3%
IGFBP-3	1.4%	4.7%
IGFBP-5	1.8%	6.9%
IGFBP-6	1.2%	7.9%
IGFBP-7	2.0%	15.6%

ASSAY CHARACTERISTICS (Continued)

Accuracy

Spike Recovery: The data was generated from mean percent recovery of 3 levels of spiked standard in five mouse serum/plasma samples.

Analyte	% Recovery in Serum/Plasma
IGFBP-1	119
IGFBP-2	94
IGFBP-3	105
IGFBP-5	167
IGFBP-6	110
IGFBP-7	114

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Insufficient Bead Count	Plate Washer aspirate height set too low	Adjust aspiration height according to manufacturers instructions.
	Bead mix prepared inappropriately	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting this into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with Alcohol flush, Back flush and washes; or if needed probe should be removed and sonicated.
	Probe height not adjusted correctly	When reading the assay on Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 4 alignment discs. When reading the assay on FLEXMAP 3D™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 1 alignment disc. When reading the assay on MAGPIX, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 2 alignment disc.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately, and pipeting with Multichannel pipets without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for cross reacting components (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes.
Beads not in region or gate	Luminex not calibrated correctly or recently	Calibrate Luminex based on Instrument Manufacturer's instructions, at least once a week or if temperature has changed by >3^{o}C.
	Gate Settings not adjusted correctly	Some Luminex instruments (e.g. Bioplex) require different gate settings than those described in the Kit protocol. Use Instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex 4 times to rid of air bubbles, wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.

	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.
Signal for whole plate is same as background	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Streptavidin-Phycoerythrin was not added	Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been removed, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been removed prior to adding Streptavidin Phycoerythrin	May need to repeat assay if desired sensitivity not achieved.
	Incubations done at inappropriate temperatures, timings or agitation	Assay conditions need to be checked.
Signals too high, standard curves are saturated	Calibration target value set too high	With some Luminex Instrument (e.g. Bio-plex) Default target setting for RP1 calibrator is set at High PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with standard curve and samples	Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Check with tech support for appropriate protocol modifications.
	Samples contain analyte concentrations higher than highest standard point.	Samples may require dilution and reanalysis for just that particular analyte.
	Standard curve was saturated at higher end of curve.	See above.
High Variation in samples and/or standards	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm all reagents are removed completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without causing splashing.
	Cross well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.
FOR FILTER PLATES ONLY		
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay setup and use supernatant.

	High lipid concentration	After centrifugation, remove lipid layer and use supernatant.
Plate leaked	Vacuum Pressure too high	Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (blocked) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate holder or raised edge so bottom of filter is not touching any surface.
	Insufficient blotting of filter plate bottom causing wicking	Blot the bottom of the filter plate well with absorbent towels after each wash step.
	Pipette touching plate filter during additions	Pipette to the side of plate.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
	Sample too viscous	May need to dilute sample.

REPLACEMENT REAGENTS **Catalog #**

Mouse IGFBP Standard	MIGFBP-8043
Mouse IGFBP Quality Controls 1 and 2	MIGFBP-6043
Serum Matrix	MIGF-SM
Mouse IGFBP Detection Antibodies	MIGFBP-1043
Streptavidin-Phycoerythrin	L-SAPE
Assay Buffer	LE-ABGLP
Set of two 96-Well Black Plates with Sealers	MAG-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Beads

	Bead #	Cat. #
IGFBP-1	27	MIGFBP1-MAG
IGFBP-2	39	MIGFBP2-MAG
IGFBP-3	42	MIGFBP3-MAG
IGFBP-5	55	MIGFBP5-MAG
IGFBP-6	61	MIGFBP6-MAG
IGFBP-7	72	MIGFBP7-MAG

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WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 0 (Background)	Standard 4	QC-1 Control	Etc.								
B	Standard 0 (Background)	Standard 4	QC-1 Control									
C	Standard 1	Standard 5	QC-2 Control									
D	Standard 1	Standard 5	QC-2 Control									
E	Standard 2	Standard 6	Sample 1									
F	Standard 2	Standard 6	Sample 1									
G	Standard 3	Standard 7	Sample 2									
H	Standard 3	Standard 7	Sample 2									