MILLIPLEX™ MAP

HUMAN IGF BINDING PROTEIN (IGFBP) PANEL KIT
96 Well Plate Assay

HIGFBP-53K

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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.

Using Luminex® xMAP® technology, Millipore has developed the MILLIPLEX MAP Human Insulin-like Growth Factor Binding Protein (IGFBP) Multiplex Panel, for the simultaneous quantification of IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, IGFBP-6, and IGFBP-7 in human serum and plasma samples, and cell culture conditioned-media samples. 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.

Millipore's MILLIPLEX™ MAP Human 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 seven different IGFBPs.
  - Choose any combination of analytes from our panel of 7 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.

Serum and plasma samples should be diluted 1:25 in the supplied Assay Buffer, prior to performing the assay. For cell culture conditioned-media samples, the samples should remain neat (non-diluted).

This kit is for research purposes only.

Please read entire protocol before use.

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

PRINCIPLE

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

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

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

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- Once the standards and controls have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE 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
Human IGFBP Standard	HIGFBP-8053	lyophilized	1 vial
Human IGFBP Quality Controls 1 and 2	HIGFBP-6053	lyophilized	2 vials
Serum Matrix Note: Contains 0.08% Sodium Azide	LSM	lyophilized	1 vial
Set of one 96-Well Filter Plate with 2 Sealers	MX-PLATE	----------	1 plate 2 sealers
Assay Buffer	LE-ABGLP2	30 mL	2 bottles
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	1 bottle
Human IGFBP Detection Antibodies	HIGFBP-1053	5.5 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE	5.5 mL	1 bottle
Bead Diluent	LBD-2	3.5 mL	1 bottle
Mixing Bottle	----------	----------	1 bottle

Human IGFBP Antibody-Immobilized Beads:

Bead/Analyte Name	Luminex Bead Region	Customizable 7 Analytes (20X concentration, 200 µL) Available	Cat. #
Anti-Human IGFBP-1 Bead	3	□	HIGFBP1
Anti-Human IGFBP-2 Bead	6	□	HIGFBP2
Anti-Human IGFBP-3 Bead	9	□	HIGFBP3
Anti-Human IGFBP-4 Bead	21	□	HIGFBP4
Anti-Human IGFBP-5 Bead	23	□	HIGFBP5
Anti-Human IGFBP-6 Bead	26	□	HIGFBP6
Anti-Human IGFBP-7 Bead	64	□	HIGFBP7

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex Sheath Fluid (Luminex Corporation, catalog #40-50000)

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. Absorbent Pads
7. Laboratory Vortex Mixer
8. Sonicator (Branson Ultrasonics Corporation, Cleaner Model #B200, or equivalent)
9. Titer Plate Shaker (Labline Scientific Instruments, Model #4625, or equivalent)
10. Vacuum Filtration Unit (Millipore Corporation, Vacuum Manifold, Catalog #MSVMHTS00, or equivalent; Vacuum Pump, Catalog #WP6111560, or equivalent)
11. Luminex 100™ IS, 200™, HTS (Luminex Corporation).
12. Plate Stand (Millipore Corporation, Catalog # MX-STAND)

SAFETY PRECAUTIONS

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

TECHNICAL GUIDELINES

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

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with 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.
- The bottom of the Microtiter Filter Plate should not come into direct contact with any surface during assay set-up or incubation times. The plate can be set on a plate stand or on the non-flat side of the plate cover, or any other plate holder to raise the

plate from any surface. A plate stand can be purchased separately from Millipore. (Millipore Corporation, Catalog #MX-STAND).

- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- After the wash steps, keep the bottom of the Microtiter Filter Plate clean by blotting on paper towels or absorbent pads to prevent any leakage due to capillary action.
- Keep the vacuum suction on the plate as low as possible. It is recommended to have a vacuum setting that will remove 200 µL of buffer in $\geq$ 5 seconds (equivalent to < 100 mmHg).
- 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 working standards, 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. In addition, adjust the probe height according to the protocols recommended by Luminex Corporation. Briefly, adjust to the kit filter plate using 3 Alignment discs prior to reading the plate.
- 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. Then, calculated sample values should be multiplied by 25 to determine the actual concentration of each IGFBP within the undiluted samples.

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. Then, calculated sample values should be multiplied by 25 to determine the actual concentration of each IGFBP within the undiluted samples.

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, and then vortex for 1 minute. Next, add 150 µL of each antibody-bead to the Mixing Bottle and bring the final volume within the Mixing Bottle to 3.0 mL with Bead Diluent. 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.

Example 1: when using 5 antibody-immobilized beads, add 150 µL from each of the five bead sets to the mixing bottle. Then add 2.25 mL of Bead Diluent.

Example 2: when using 7 antibody-immobilized beads, add 150 µL from each of the seven bead sets to the mixing bottle. Then add 1.95 mL of Bead Diluent.

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

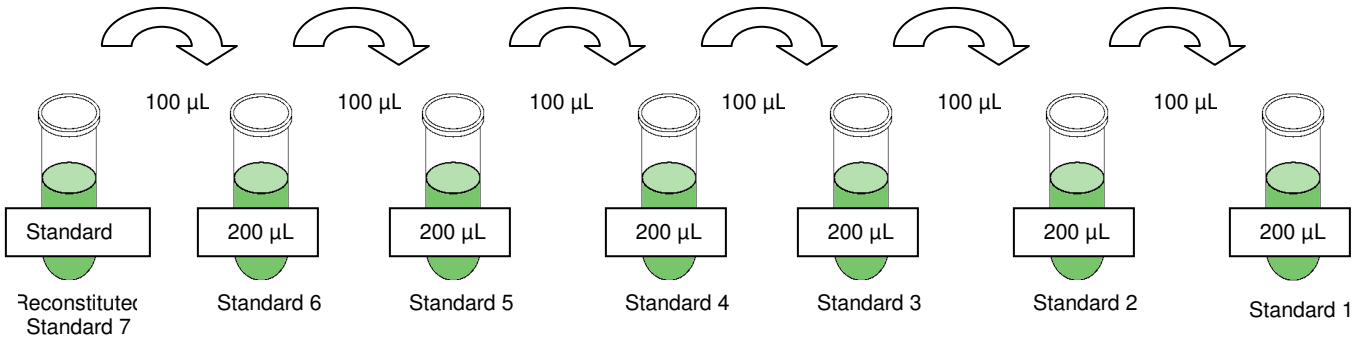
This step is required for serum or plasma samples only.

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

E. Preparation of Human IGFBP Standard

Prior to use, reconstitute the Human IGFBP Standard ("Standard 7") with 250 µL deionized water, invert the vial several times to mix, and allow the vial to sit for 5 minutes. Then, vortex the vial and transfer the contents to a polypropylene microfuge tube labeled "Standard 7". Also, label six polypropylene microfuge tubes "Standard 6", "Standard 5", "Standard 4", "Standard 3", "Standard 2", and "Standard 1", and add 200 µL of Assay Buffer to each of the six tubes. Perform 3-fold 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



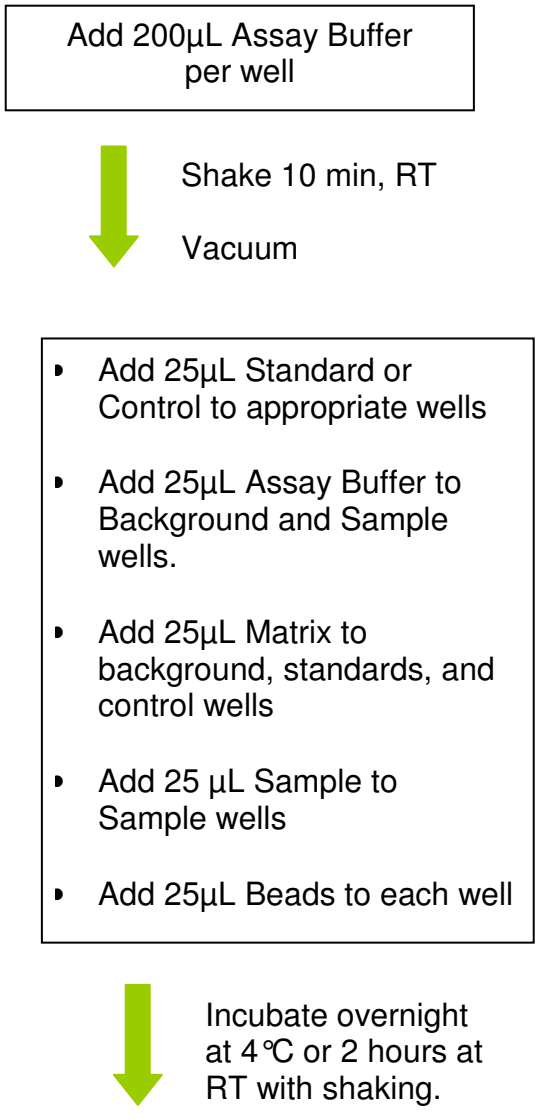
After dilution, the tubes should have the following concentrations for constructing standard curves.

Standard tube	IGFBP-1 (ng/mL)	IGFBP-2 (ng/mL)	IGFBP-3 (ng/mL)	IGFBP-4 (ng/mL)	IGFBP-5 (ng/mL)	IGFBP-6 (ng/mL)	IGFBP-7 (ng/mL)
Standard 1	0.03	0.34	0.34	0.69	1.37	0.14	0.07
Standard 2	0.10	1.03	1.03	2.06	4.12	0.41	0.21
Standard 3	0.31	3.09	3.09	6.17	12.35	1.23	0.62
Standard 4	0.93	9.26	9.26	18.52	37.04	3.70	1.85
Standard 5	2.78	27.78	27.78	55.56	111.11	11.11	5.56
Standard 6	8.33	83.33	83.33	166.67	333.33	33.33	16.67
Standard 7	25.00	250.00	250.00	500.00	1000.00	100.00	50.00

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.
- 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. Block the filter plate by pipetting 200 µL of Assay Buffer into each well of the microtiter plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Remove Assay Buffer by vacuum. **(NOTE: DO NOT INVERT PLATE)**. Blot excess Assay Buffer from the bottom of the plate with an absorbent pad or paper towels.
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, and cover it with the lid. Then, wrap a rubber band around the plate holder, plate, and lid, and incubate with agitation on a plate shaker for overnight (16-18 hours) incubation at 4°C or 2 hours at room temperature (20-25°C). *An overnight incubation may improve assay sensitivity for some analytes.*



9. Gently remove fluid by vacuum. **(NOTE: DO NOT INVERT PLATE)**.
10. Wash plate 3 times with 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Blot excess Wash Buffer from the bottom of the plate with an absorbent pad or paper towels.
11. Add 50 µL of Detection Antibodies into each well. (Note: allow the Detection Antibodies to warm to room temperature prior to addition.)
12. Seal, cover with lid, and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C)

    NOTE: DO NOT VACUUM AFTER THIS INCUBATION
13. Add 50 µL Streptavidin-Phycoerythrin to each well containing the 50 µL of Detection Antibodies.
14. Seal, cover with lid, and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
15. Gently remove all contents by vacuum. **(NOTE: DO NOT INVERT PLATE)**.
16. Wash plate 3 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Wipe any excess buffer on the bottom of the plate with a tissue.
17. Add 100 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.
18. Run plate on Luminex 100™ IS, 200™, HTS.
19. 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: As stated in the "Sample Collection and Storage" section, serum and plasma samples should be diluted 25-fold in Assay Buffer prior to performing this assay. Therefore, multiply the IGFBP concentrations of the diluted samples by 25 to determine the concentrations within the undiluted samples.

Vacuum and wash 3X with 200µL Wash Buffer

Add 50µL Detection Antibody per well

Incubate for 30 minutes at RT

Do Not Vacuum

Add 50 µL Streptavidin-Phycoerythrin per well

Incubate for 30 minutes at RT

Vacuum and wash 3X with 200µL Wash Buffer

Add 100µL Sheath Fluid per well

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

EQUIPMENT SETTINGS

These specifications are for the Luminex 100™ IS v.1.7 or Luminex 100™ IS v2.1/2.2, Luminex 200™ v2.3, xPONENT, and Luminex HTS. Luminex instruments with other software (e.g. MasterPlex, StarStation, LiquiChip, Bio-Plex, LABScan100) would need to follow instrument instructions for gate settings and additional specifications from the vendors.

Events:	50, per bead	
Sample Size:	50 µL	
Gate Settings	8,000 to 15,000	
Time Out	60 seconds	
Bead Set:	7-Plex Beads	
	IGFBP-1	3
	IGFBP-2	6
	IGFBP-3	9
	IGFBP-4	21
	IGFBP-5	23
	IGFBP-6	26
	IGFBP-7	64

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 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 pg/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	Overnight Protocol, Serum Matrix (n= 6 assays)		1-Day Protocol, Serum Matrix (n= 2 assays)	
	Min DC	Min DC + 2SD	Min DC	Min DC + 2SD
IGFBP-1	0.013	0.017	0.016	0.02
IGFBP-2	0.325	0.444	0.565	0.607
IGFBP-3	0.145	0.178	0.130	0.130
IGFBP-4	0.573	0.776	0.675	0.689
IGFBP-5	1.15	1.6	0.85	1.3
IGFBP-6	0.078	0.093	0.120	0.148
IGFBP-7	0.044	0.062	0.045	0.069

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentrations of IGFBP in one experiment. Inter-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentrations of IGFBP in 3 different experiments.

Intra-assay: $\leq$ 10%

Inter-assay: < 18%

Accuracy

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

Analyte	Overnight Protocol	1-Day Protocol
	% Recovery in Serum/Plasma	% Recovery in Serum/Plasma
IGFBP-1	83.1	93.2
IGFBP-2	61.5	64.9
IGFBP-3	87.0	90.7
IGFBP-4	96.8	101.6
IGFBP-5	149.4	118.9
IGFBP-6	83.6	93.8
IGFBP-7	95.5	98.3

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds
	Samples have insoluble particles	Centrifuge samples just prior to assay setup and use supernatant If high lipid concentration, after centrifugation, remove lipid layer and use supernatant.
	Sample too viscous	May need to dilute sample
Insufficient Bead Count	Vacuum pressure too high	Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds
	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	Adjust probe to 3 alignment discs in well H6.
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.
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 vacuumed out, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been vacuumed out 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 vacuumed out 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.

REPLACEMENT REAGENTS	**Catalog #**
Human IGFBP Standard	HIGFBP-8053
Human IGFBP Quality Controls 1 and 2	HIGFBP-6053
Serum Matrix	LSM
Human IGFBP Detection Antibodies	HIGFBP-1053
Bead Diluent	LBD-2
Streptavidin-Phycoerythrin	L-SAPE
Assay Buffer	LE-ABGLP2
Set of two 96-Well Filter Plates with Sealers	MX-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Beads

	Bead #	Cat. #
IGFBP-1	3	HIGFBP1
IGFBP-2	6	HIGFBP2
IGFBP-3	9	HIGFBP3
IGFBP-4	21	HIGFBP4
IGFBP-5	23	HIGFBP5
IGFBP-6	26	HIGFBP6
IGFBP-7	64	HIGFBP7

ORDERING INFORMATION

To place an order:

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

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

FAX: (636) 441-8050
Toll Free US: (866) 441-8400
(636) 441-8400

MAIL ORDERS: Millipore Corp.
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

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

Conditions of Sale

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

Material Safety Data Sheets (MSDS)

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

WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 0 (Background)	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									