MILLIPLEX™ MAP

HUMAN IGF-1 SINGLE PLEX KIT
96 Well Plate Assay

HIGF1-52K-01

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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION

Insulin-like Growth Factor (IGF-1) is a polypeptide endocrine hormone that is similar in structure to insulin. Stimulated by growth hormone and produced primarily in the liver, it binds to the IGF-1 receptor with high affinity and to the insulin receptor with less affinity. The IGF-1 receptor, a tyrosine kinase receptor found on cell types in many tissues, is a potent activator of the Akt signaling pathway, which drives a variety of processes including cell growth and division and apoptosis inhibition. Increased levels of IGF-1 have been correlated with an increased risk of cancer. In addition, while it appeared to show great promise in reducing hemoglobin A1C levels and daily insulin consumption in types 1 and 2 diabetes, the hormone has been implicated in the development of diabetic retinopathy. Drugs have been developed as replacement therapy for severe primary IGF-1 deficiency, a group of diseases in which patients do not respond to growth hormone therapy.

IGF-1 is involved in a wide range of therapeutic areas including diabetes, growth deficiency, and cancer. Millipore recognizes the significance of studying this endocrine hormone and, therefore, announces the launch of the MILLIPLEX MAP Human IGF-1 Single Plex. This single plex provides biomedical researchers with a quality tool for the study of today's crippling diseases.

Millipore's MILLIPLEX™ Human IGF-1 kit is a one-plex assay kit, manufactured by Millipore, to be used for the quantitative determination of IGF-1 in human serum, plasma, tissue/cell extracts, or culture media samples. Serum and plasma samples require an extraction step resulting in a 1:8 final dilution.

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 is 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.

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 Antibody, and Streptavidin-Phycoerythrin**.

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8 °C

REAGENTS SUPPLIED	CATALOG NUMBER	VOLUME	QUANTITY
Human IGF-1 Standard	HIGF1-8052	lyophilized	1 vial
Human IGF-1 Quality Controls 1 and 2	HIGF1-6052	lyophilized	2 vials
Set of one 96-Well Filter Plates with 2 sealers	MX-PLATE	----------	1 Plate 2 Sealers
Assay Buffer	L-AB2	30 mL	1 bottle
Activation Buffer Note: Contains 87.5% Ethanol and 0.25N HCl	L-ATVB	20 mL	1 bottles
Neutralization Buffer	L-NAB	10 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	1 bottle
Human IGF-1 Detection Antibody	HIGF1-1052	5.5 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE5	5.5 mL	1 bottle
Mixing Bottle	----------	----------	1 bottle
Human IGF-1 Antibody-Immobilized Beads (20x concentration), Bead# 02	HIGF1	200 µL	1 vial

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex Sheath Fluid (Luminex Catalogue #40-50000)

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
2. Multichannel Pipettes capable of delivering 5 µl to 50 µL or 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 Ultrasonic Cleaner Model # B200 or equivalent)
9. Titer Plate Shaker (Lab-Line Instruments, Model #4625, or equivalent)
10. Vacuum Filtration Unit (Millipore Vacuum Manifold Catalog #MSVMHTS00, or equivalent. Millipore Vacuum Pump Catalog #WP6111560 or equivalent.)
11. Luminex$^{100,\ 200,\ HTS}$ by Luminex Corporation
12. Plate Holder (Millipore 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.
- Activation Buffer L-ATVB contains ethanol and HCl. Use in a well ventiliated area.

TECHNICAL GUIDELINES

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

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- The bottom of the Microtiter Filter Plate should not be in direct contact with any surface during assay set-up or incubation times. The plate can be set on a plate holder or on the non-flat side of the plate cover or any other plate holder to raise the plate from the surface. The plate holder can be purchased separately from Millipore. (Millipore 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 which may be stored at ≤ -20°C for 1 month and at ≤ -80°C for greater than one month.

- 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 Antibody-Immobilized Beads may be stored in the Bead Mix bottle at 2-8°C for up to one month.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for the analyte.
- 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. Adjust probe height according to the protocols recommended by Luminex to the kit filter plate using 3 Alignment discs prior to reading an assay.
- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in blank, standard curve and controls. If samples are diluted in assay buffer, use the assay buffer as matrix.
- For cell/tissue homogenate, 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. Avoid debris, lipids, and cell/tissue chunks. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000 xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20ºC.
- 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.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20ºC.
- 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.

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ -20ºC.
- Avoid multiple (>2) freeze/thaw cycles.
- Tissue Culture Supernatant may require a dilution with an appropriate control medium prior to assay.

SAMPLE EXTRACTION PROCEDURE

Mix 100 µL serum or plasma with 400 µL Activation Buffer and mix well. Let sit for 30 minutes at room temperature. Centrifuge for 5 minutes at 10000 xg. Carefully transfer 250 µL supernatant into a microfuge tube and add 150 µL Neutralization Buffer. Mix well. During the extraction process, the sample is 1:8 diluted.

NOTE:

- A maximum of 25 µL per well extracted serum or plasma can be used. Tissue culture or other media may also be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- **Activation Buffer bottle should remain closed when not in use to prevent evaporation of ethanol.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anticoagulant, since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

A 25 µL per well of extracted serum or plasma will be used. The sample dilution of tissue/cell extracts and culture media may vary.

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

Sonicate antibody-bead vial for 30 seconds; vortex for 1 minute. Add 150 µL from antibody bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Assay Buffer as the bead diluent. Vortex the mixed beads well. Unused portions may be stored at 2-8°C for up to one month.

B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL Deionized Water. Invert the vial several times to mix and vortex. Allow the vial 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 Human IGF-1 Standard

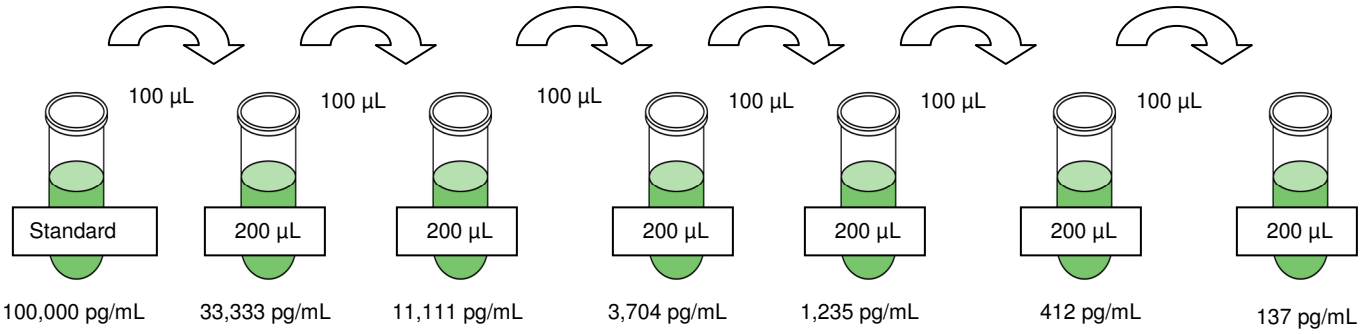
1.) Prior to use, reconstitute the Human IGF-1 Standard with 250 µL Deionized Water to give a 100,000 pg/mL concentration of standard for IGF-1. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes and then transfer the standard to appropriately labeled polypropylene microfuge tube. This will be used as the 100,000 pg/mL standard; the unused portions may be stored at ≤ -20°C for up to one month.

2.). Preparation of Working Standards

Label six polypropylene microfuge tubes 33,333, 11,111, 3,704, 1,235, 412, and 137 pg/mL. Add 200 µL of Assay Buffer to each of the six tubes. Prepare 3 times serial dilutions by adding 100 µL of the 100,000 pg/mL reconstituted standard to the 33,333 pg/mL tube, mix well and transfer 100 µL of the 33,333 standard to the 11,111 pg/mL tube, mix well and transfer 100 µL of the 11,111 standard to the 3,704 pg/mL tube, mix well and transfer 100 µL of the 3,704 standard to 1,235 pg/mL tube, mix well and transfer 100 µL of the 1,235 pg/mL standard to the 412 pg/mL tube, mix well and transfer 100 µL of the 412 pg/mL standard to the 137 pg/ml tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Standard Concentration (pg/mL)	Volume of Deionized Water to Add	Volume of Standard to Add
100,000	250 µL	0

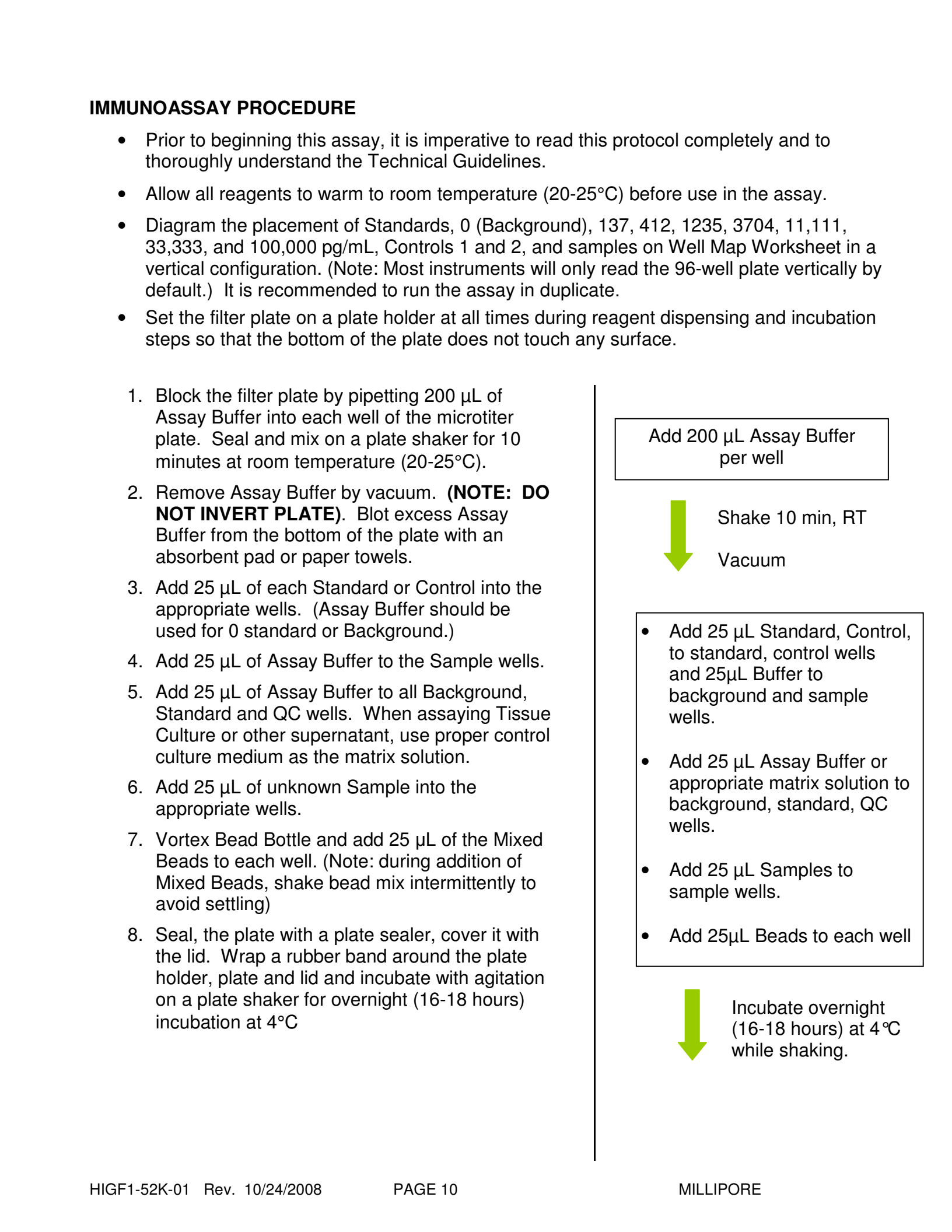
Standard Concentration (pg/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
33,333	200 µL	100 µL of 100,000 pg/mL
11,111	200 µL	100 µL of 33,333 pg/mL
3,704	200 µL	100 µL of 11,111 pg/mL
1,235	200 µL	100 µL of 3,704 pg/mL
412	200 µL	100 µL of 1,235 pg/mL
137	200 µL	100 µL of 412 pg/mL



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), 137, 412, 1235, 3704, 11,111, 33,333, and 100,000 pg/mL, 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 0 standard or Background.)
4. Add 25 µL of Assay Buffer to the Sample wells.
5. Add 25 µL of Assay Buffer to all Background, Standard and QC wells. When assaying Tissue Culture or other supernatant, use proper control culture medium as the matrix solution.
6. Add 25 µL of unknown Sample into the appropriate wells.
7. Vortex Bead Bottle and add 25 µL of the Mixed Beads to each well. (Note: during addition of Mixed Beads, shake bead mix intermittently to avoid settling)
8. Seal, the plate with a plate sealer, cover it with the lid. 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



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 the plate by 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)
13. **DO NOT VACUUM AFTER INCUBATION**
14. Add 50 µL Sreptavidin-Phycoerythrin to each well containing the 50 µL of Detection Antibodies.
15. Seal, cover with lid and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
16. Gently remove all contents by vacuum. **(NOTE: DO NOT INVERT PLATE)**.
17. 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.
18. Add 100 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.
19. Run plate on Luminex$^{100, 200, HTS}$.
20. Save and analyze the median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating IGF-1 concentrations in samples. Multiply concentration values by a dilution factor of 8.

Vacuum. Wash 3X with 200µL Wash Buffer

Add 50 µL Detection Antibody per well

Incubate 30 min at RT

Do Not Vacuum

Add 50 µL Streptavidin-Phycoerythrin per well

Incubate for 30 minute at RT

Vacuum and Wash 3X with 200µL Wash Buffer

Add 100 µL Sheath Fluid per well

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

EQUIPMENT SETTINGS

These specifications are for the Luminex^{100} v.1.7, Luminex100IS v2.1/2.2, Luminex^{200} v2.3, xPONENT®, and Luminex^{HTS}. Luminex instruments with other software (e.g. MasterPlex®, StarStation®, LiquiChip®, Bio-Plex®, LABScan®100) 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	
Reporter Gain:	Default (low PMT)	
Time Out	60 seconds	
Bead Set:	IGF-1	02

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

Assay Sensitivities (minimum detectable concentrations, pg/mL)

Minimum Detectable concentration (MinDC) defines the reportable range of the assay. It is a measure of the true limits of detection for an assay and is mathematically determined.

Analyte	Overnight Protocol (N = 8 assays)	
	Mean MinDC	Mean MinDc + 2SD
IGF-1	52	68

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentration of IGF-1 in one experiment. Inter-assay precision is generated from the mean of the %CV's from two reportable results each for two different concentrations of IGF-1 across 4 different experiments.

Analyte	Intra-Assay (CV%)	Inter-Assay (CV%)
IGF-1	4	7

Accuracy

The data represents mean recovery of 3 levels of spiked standards ranging from 1,235 pg/ml to 11,111 pg/mL in extracted human serum samples in 8 independent experiments.

Analyte	Spike Recovery
IGF-1	84%

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 pipettes
	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	**Cat #**
Human IGF-1 Standard	HIGF1-8052
Human IGF-1 Quality Controls	HIGF1-6052
Human IGF-1 Detection Antibody	HIGF1-1052
SAPE	L-SAPE5
Activation Buffer	L-ATVB
Neutralization Buffer	L-NAB
Assay Buffer	L-AB2
Set of two 96-Well Filter Plates with sealers	MX-PLATE
10X Wash Buffer	L-WB
IGF-1 Antibody-Immobilized Beads (Region # 02)	HIGF1

ORDERING INFORMATION

To place an order:

To assure the clarity of your custom 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

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	0 pg/mL Standard (Background)	3,704 pg/mL Standard	QC-1 Control	Etc.								
B	0 pg/mL Standard (Background)	3,704 pg/mL Standard	QC-1 Control									
C	137 pg/mL Standard	11,111 pg/mL Standard	QC-2 Control									
D	137 pg/mL Standard	11,111 pg/mL Standard	QC-2 Control									
E	412 pg/mL Standard	33,333 pg/mL Standard	Sample 1									
F	412 pg/mL Standard	33,333 pg/mL Standard	Sample 1									
G	1,235 pg/mL Standard	100,000 pg/mL Standard	Sample 2									
H	1,235 pg/mL Standard	100,000 pg/mL Standard	Sample 2									