

# Cancer Biomarker Panel (MIF, Leptin, Prolactin, OPN, CA-125, IGF-II)

Cat. # 48-020

# MILLIPLEX<sup>®</sup> MAP

# Cancer Biomarker Panel Kit (MIF, Leptin, Prolactin, OPN, CA-125, IGF-II)

#### #48-020

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

## INTRODUCTION

Often called "the disease that whispers", ovarian cancer symptoms often go unnoticed and, therefore, undiagnosed until a patient has developed advanced disease. In collaboration with Millipore, Dr. Gil Mor of Yale University identified and characterized six serum biomarkers able to discriminate between disease-free and ovarian cancer patients, even early Stages I and II, with high sensitivity and accuracy, using a unique algorithm developed by his Laboratory. In addition to the importance of these biomarkers in ovarian cancer, many of them have been detected in other tumors such as breast, colorectal, lung and prostate cancers. The biomarkers represented in this panel demonstrate the significance of assessing biomarker concentrations that are affected by the body's response to the presence of cancer cells.

Millipore proudly offers the MILLIPLEX<sup>®</sup> MAP Human Cancer Biomarker Panel. This Panel includes both a Five-Plex assay, which simultaneously measures five serum biomarkers, Macrophage Migration Inhibitory Factor (MIF), Prolactin, CA-125, Leptin and Osteopontin (OPN), and a Single Plex IGF-II assay. The Human Cancer Biomarker Panel is a valuable tool for researchers studying the biochemical mechanisms and signals of early stage cancer.

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<sup>®</sup> 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 controls have been reconstituted, immediately transfer contents into polypropylene vials. DO NOT STORE RECONSITUTED STANDARDS AND CONTROLS IN GLASS VIALS. For long-term storage, freeze reconstituted standards and controls at ≤ -80°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
Cancer Biomarker Panel Standard 5	47-033	lyophilized	1 vial
Cancer Biomarker Panel Standard 1	47-035	lyophilized	1 vial
Cancer Biomarker 5, Quality Control 1 and 2	47-036	lyophilized	2 vials
Cancer Biomarker 1, Quality Control 1 and 2	47-037	lyophilized	2 vials
Serum and Standard Diluent (SSD)	43-050	15 mL	1 vial
Assay Buffer	43-051	50 mL	1 bottle
IGF-II Activation Buffer	43-053	20 mL	1 bottle
IGF-II Neutralization Buffer	43-054	10 mL	1 bottle
Set of two 96-Well MultiScreen HTS Filter Plates with four Plastic Sealers	L-PLATE		2 plates 4 sealers
Cancer Biomarker Panel Beads 5	42-077	3.5 mL	1 bottle
Cancer Biomarker Panel Beads 1	42-078	3.5 mL	1 bottle
Cancer Biomarker Panel Biotin 5	44-077	5.5 mL	1 bottle
Cancer Biomarker Panel Biotin 1	44-078	5.5 mL	1 bottle
Streptavidin-Phycoerythrin	45-002	5.5 mL	2 bottles
10X Concentrated Wash Buffer	43-052	30 mL	2 bottles

#### Cancer Biomarker Panel Antibody-Immobilized Beads:

Bead/Analyte Name	Luminex Bead Region
Anti-MIF Bead	12
Anti-Prolactin Bead	24
Anti-CA-125 Bead	25
Anti-Leptin Bead	28
Anti-IGF-II Bead	29
Anti-Osteopontin Bead	30

# MATERIALS REQUIRED BUT NOT PROVIDED

## **Reagents**

• Luminex Sheath Fluid (Luminex Catalog #40-50000)

## **Instrumentation / Materials**

- Pipettes (single and multi-channel) capable of delivering 25 µL to 1000 µL
- Reagent Reservoirs
- Polypropylene Microfuge Tubes
- Rubber Bands
- Aluminum Foil
- Absorbent Pads
- Laboratory Vortex Mixer
- Sonicator
- Titer Plate Shaker
- Vacuum Filtration Unit (Millipore Vacuum Manifold Catalog #MSVMHTS00 or equivalent with Millipore Vacuum Pump Catalog #WP6111560 or equivalent)
- Luminex 100<sup>™</sup> IS, 200<sup>™</sup>, or HTS by Luminex Corporation
- Plate Stand (Millipore Catalog # MX-STAND)

# SAFETY PRECAUTIONS

- All tissue 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 be in 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 the surface. A plate stand 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 appropriate Assay 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 ≥ 5 seconds (equivalent to < 100 mmHg).</li>
- Any unused mixed Antibody-Immobilized Beads may be stored in the Mixing Bottle at 2-8°C for up to one month.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity.
- 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.
- Vortex all reagents well before adding to plate.

# SAMPLE PREPARATION AND STORAGE

For measurement of MIF, Leptin, Prolactin, CA-125 and OPN: Vortex samples and dilute in Serum and Standard Diluent (catalog # 43-050). It is recommended to make a 1:6 dilution for samples. Vortex thoroughly.

For measurement of IGF-II: Serum and plasma samples must be activated prior to assay. It is recommended that Steps 2-4 of Assay Protocol on page 10 be performed during the one hour incubation. Vortex samples and dilute with IGF-II Activation Buffer (catalog # 43-053). It is recommended to make a 1:11 dilution for samples (i.e. 20  $\mu$ L of sample added to 200  $\mu$ L Activation Buffer). Vortex thoroughly. Incubate for 60 minutes at 37°C in water bath. Centrifuge samples for 6 minutes at 1,000 rpm using a microfuge. Transfer 100  $\mu$ L of supernatant to a new tube. Add 100  $\mu$ L of IGF-II Neutralization Buffer to each activated sample and vortex. This step must be performed immediately prior to assay (refer to page 10 step 1).

# PREPARATION OF REAGENTS

# A. Preparation of antibody capture beads

MILLIPLEX<sup>®</sup> MAP Cancer Biomarker Panel, Beads 5 and Cancer Biomarker Panel, Beads 1 are provided as a 1X stock and should be protected from light. Sonicate the premixed bead bottles 30 seconds and then vortex for 1 minute before use.

#### B. Preparation of 10X 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 portion at 2-8°C for up to one month.

# C. Preparation of Quality Controls

Reconstitute Cancer <u>Biomarker 5</u> Quality Controls 1 and 2 each with 0.5 mL Serum and Standard Diluent (Cat # 43-050). 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 microfuge tubes. Unused portion may be stored at  $\leq$  -20°C for up to one month.

Reconstitute Cancer <u>Biomarker 1</u> Quality Controls 1 and 2 each with 0.5 mL Assay Buffer (catalog # 43-051). 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 microfuge tubes. Unused portion may be stored at  $\leq$  -20°C for up to one month.

# D. Preparation of Standards

Reconstitute Cancer Biomarker Panel <u>Standard 5</u> with 0.5 mL Serum and Standard Diluent (Cat # 43-050). The following concentrations will result:

Analyte	unit/vial	Final Concentration
MIF	5,000 pg	10,000 pg/mL
Prolactin	200 ng	400 ng/mL
CA-125	2,500 IU	5,000 IU/mL
Osteopontin	200 ng	400 ng/mL
Leptin	50 ng	100 ng/mL

Prepare a 1:5 serial dilution as follows.

Std	Volume of Standard	SSD (μL)	MIF	Prolactin	CA125	OPN	Leptin
			pg/mL	ng/mL	IU/mL	ng/mL	ng/mL
7		500	10,000	400	5,000	400	100
6	100 μL of Std 7	400	2,000	80	1,000	80	20
5	100 μL of Std 6	400	400	16	200	16	4
4	100 μL of Std 5	400	80	3.2	40	3.2	0.8
3	100 μL of Std 4	400	16	0.64	8	0.64	0.16
2	100 μL of Std 3	400	3.2	0.128	1.6	0.128	0.032
1	100 μL of Std 2	400	0.64	0.0256	0.32	0.0256	0.0064
0	0	400	0	0	0	0	0

Reconstitute Cancer Biomarker Panel <u>Standard 1</u> with 0.5 mL Assay Buffer (catalog # 43-051).

Analyte	unit/vial	Final Concentration
IGF-II	250 ng	500 ng/mL

Prepare a 1:3 serial dilution as follows.

Standard	Volume of Standard	Assay Buffer	IGF-II
Stanuaru		(μL)	(ng/mL)
7	-	500	500.0
6	100 μL of Std 7	200	166.7
5	100 μL of Std 6	200	55.6
4	100 μL of Std 5	200	18.5
3	100 μL of Std 4	200	6.2
2	100 μL of Std 3	200	2.1
1	100 μL of Std 2	200	0.7
0	-	200	0

# MILLIPLEX<sup>®</sup> MAP CANCER BIOMARKER PANEL ASSAY PROTOCOL FOR MULTIPLEXED DETECTION OF MIF, LEPTIN, PROLACTIN, CA-125 AND OPN

- 1. Prewet the filter plate by pipetting 100  $\mu$ L of Assay Buffer into each well of the Microtiter Filter Plate. Incubate for 10 minutes.
- Resuspend the Cancer Biomarker Panel Standard 5 (Cat # 47-033) in 0.5 mL Serum and Standard Diluent and vortex on medium speed for 15 seconds. Place on ice for 5 minutes. Perform 1:5 serial dilution as shown on page 7.
- Resuspend the Cancer Biomarker 5 Quality Controls 1 & 2 (Cat # 47-036) in 0.5 mL Serum and Standard Diluent and vortex on medium speed for 15 seconds. Place on ice for 5 minutes.
- 4. Apply vacuum to bottom of plate to remove liquid in wells. Blot bottom of plate on paper towels.
- 5. Add 50  $\mu$ L standard, 50  $\mu$ L quality controls and 50  $\mu$ L of diluted serum/plasma samples to respective wells in duplicate.
- Vortex each of the Cancer Biomarker Panel Beads 5 (Cat # 42-077) at medium speed for 15 seconds and then sonicate for additional 15 seconds using a sonication bath. Add 25 μL of bead solution to each well.
- Cover the plate with a plastic sealer. Incubate overnight (16-18 hours) in the dark at 4°C with moderate shaking on a plate shaker.
- 8. Move the reagents to room temperature. Warm the plate on a plate shaker at room temperature for 1 hour.
  - Apply vacuum to bottom of filter plate to remove liquid in wells. Wash plate 2 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 with an absorbent pad or paper towels.
- 10. Add 50 μL Cancer Biomarker Panel Biotin 5 (Cat # 44-077) to each well.
- 11. Cover the plate with the plastic sealer. Incubate for 1.5 hours in the dark at room temperature with shaking on a plate shaker.

Add 100 µL Assay Buffer per well.



Resuspend the Standard 5 in 0.5 mL Serum and Standard Diluent and make serial dilutions. Resuspend Quality Controls 1&2 in 0.5 mL Serum and Standard Diluent.



Vacuum Filter Plate

Add 50  $\mu$ L standard, quality controls or diluted samples to each well, add 25  $\mu$ L bead mix.



Incubate 4°C overnight in dark with shaking

Warm reagents & plate at RT for 1 hour. Vacuum & Wash 2X with 200 µL Wash Buffer.



Add 50 µL Biotin 5 per well.



Incubate at RT for 1.5 hours in the dark with shaking

- Do not vacuum plate. Add 50 μL Streptavidin-Phycoerythrin (Catalog # 45-002) directly to each well containing 50 μL Cancer Biomarker Panel, Biotin 5.
- 13. Cover the plate with the plastic sealer. Incubate exactly 30 minutes in the dark at room temperature with shaking on a plate shaker.
- 14. Apply vacuum to bottom of filter plate to remove liquid in wells. Wash plate 2 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 with an absorbent pad or paper towels.
- 15. Add 125  $\mu$ L / well of sheath fluid, cover and shake the plate for 1-5 minutes.
- 16. Read on Luminex<sup>®</sup> 100<sup>™</sup> IS, 200<sup>™</sup>, HTS.
- Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating concentrations in samples. (Note: For diluted samples, multiply the calculated concentration by the dilution factor.)

Add 50 µL SA-PE per well.



Incubate for exactly 30 minutes at RT in the dark with shaking

Vacuum and wash 2X with 200  $\mu$ L of Wash Buffer & resuspend in 125  $\mu$ L of sheath fluid, shake 1-5 minutes.

Read results on Luminex<sup>®</sup>

# MILLIPLEX<sup>®</sup> MAP CANCER BIOMARKER PANEL ASSAY PROTOCOL FOR MULTIPLEXED DETECTION OF IGF-II

- Activation of serum/plasma samples: Vortex samples and make dilution at 1:11 with IGF-II Activation Buffer (i.e. 20 μL of serum sample plus 200 μL activation buffer). Vortex thoroughly. Incubate for 60 minutes at 37°C in a water bath (during this incubation, do Steps 2 through Step 4 below). Centrifuge samples for 6 minutes at 1,000 rpm using a microfuge. Transfer 100 μL of supernatant to a new tube. Add 100 μL of IGF-II Neutralization Buffer to each activated sample, vortex and proceed to Step 6 immediately.
- 2. Wet filter plate with 100  $\mu$ L of Assay Buffer per well. Incubate for at least 10 minutes.
- Resuspend the Cancer Biomarker Panel Standard 1 (Cat # 47-035) in 0.5 mL Assay Buffer and vortex on medium speed for 15 seconds. Perform serial dilution (1:3) as shown on page 7.
- Resuspend the Cancer Biomarker 1 Quality Controls 1 and 2 (Cat # 47-037) in 0.5 mL Assay Buffer and vortex on medium speed for 15 seconds. Place on ice for 5 minutes.
- 5. Apply vacuum to bottom of plate to remove liquid in wells. Blot bottom of plate on paper towels.
- Add 50 µL of standards, quality controls, and treated samples from Step 1 to the respective wells in duplicate. Allow the plate to sit for 30 minutes.
- Vortex the Cancer Biomarker Panel Beads 1 at high speed for 15 seconds and sonicate for an additional 15 seconds using a sonication bath. Add 25 μL of bead suspension to each well.
- 8. Cover the plate with a plastic sealer. Incubate overnight (16-18 hours) in the dark at 4°C with moderate shaking on a shaker.
- 9. Move the reagents to room temperature. Warm the plate on a plate shaker at room temperature for 1 hour.
- Apply vacuum manifold to bottom of filter plate to remove liquid in wells. Wash plate 2 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 with an absorbent pad or paper towels.





Incubate 4°C overnight in dark with shaking

Warm reagents & plate at RT for 1 hour. Vacuum & Wash 2X with 200 µL Wash Buffer.

- Add 50 µL Cancer Biomarker Panel Biotin 1 (Cat # 44-078) to each well. Cover the plate with the plastic sealer. Incubate 1.5 hours in the dark at room temperature with shaking on a plate shaker.
- 12. **Do not vacuum plate**. Add 50 μL Streptavidin-Phycoerythrin (Catalog # 45-002) directly to each well containing 50 μL Cancer Biomarker Panel Biotin 1.
- 13. Cover the plate with the plastic sealer. Incubate 30 minutes in the dark at room temperature with shaking on a plate shaker.
- 14. Apply vacuum to bottom of filter plate to remove liquid in wells. Wash plate 2 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 with an absorbent pad or paper towels.
- 15. Add 125  $\mu$ L / well of sheath fluid, cover and shake the plate for 1-5 minutes.
- 16. Read on Luminex<sup>®</sup> 100<sup>™</sup> IS, 200<sup>™</sup>, HTS.
- 17. Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating concentrations in samples. Multiply concentration values by a dilution factor of 22.

Add 50 µL Biotin 1 per well.



Add 50 µL SA-PE per well.



Incubate 30 minutes at RT in the dark with shaking

Vacuum and wash 2X with 200  $\mu$ L of Wash Buffer & resuspend in 125  $\mu$ L of sheath fluid, shake 1-5 minutes.

Read results on Luminex<sup>®</sup>

# **EQUIPMENT SETTINGS**

These specifications are for the Luminex<sup>100</sup> v.1.7 or Luminex 100IS v2.1/2.2, Luminex<sup>200</sup> 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	100	μL			
Gate Settings	8,000 to <sup>-</sup>	15,000			
Reporter Gain	Default (Lo	ow PMT)			
Time Out	60 seconds				
Bead Set	MIF 12				
	Prolactin 24				
	CA-125 25				
	Leptin 28				
	IGF-II 29				
	Osteopontin	30			

#### 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**

#### Sensitivity (minimum detectable concentrations) and Assay Range

Analyte	MIF (pg/mL)	Prolactin (ng/mL)	CA-125 (IU/mL)	Leptin (ng/mL)	OPN (ng/mL)	IGF-II (ng/mL)
Sensitivity	0.4	0.0009	0.12	0.008	0.03	0.2
Assay Range	0.4-10,000	0.0009-400	0.12-5000	0.008-100	0.03-400	0.2-500

#### Precision

Analyte	MIF	Prolactin	CA-125	Leptin	OPN	IGF-II
Intra-assay CV%	6.9	10.4	7.5	5.9	9.9	8.6
Inter-assay CV%	6.8	7.1	6.8	4.5	7.5	3.4

#### Accuracy

Analyte	MIF	Prolactin	CA-125	Leptin	OPN	IGF-II
% Recovery in matrix	97.4	97.3	96.0	98.5	98.8	87.0

#### **Cross-Reactivity**

There was no significant cross-reactivity between the antibodies and any of the other analytes in this panel.

# **TROUBLESHOOTING GUIDE**

Situation	Possible Problem	Solution
	<u>Mechanical</u>	<u>Mechanical</u>
Data acquisition time exceeds 30 seconds per well and/or "Sample Empty" occurs	<ol> <li>Needle height is not correct (common problem due to variation in well depth between plate types).</li> <li>Sample needle is clogged.</li> <li>Air in the system.</li> <li>Low pressure in the system.</li> </ol>	<ol> <li>Adjust needle height using 2 disks using "options; XY setup function".</li> <li>Remove needle (see user's manual) and sonicate it to remove obstruction.</li> <li>Perform alcohol flush and then wash with alcohol; followed by a wash with sheath fluid and a prime.</li> <li><u>Tighten</u> tops on the <i>sheath fluid</i> container.</li> <li><u>Loosen</u> the top on the <i>waste</i> container.</li> </ol>
Readings are lower than expected	<ol> <li>Gate is not set properly.</li> <li>Calibration is not correct.</li> <li>Cells responded poorly to stimulation.</li> </ol>	<ol> <li>Right click within the "doublet discriminator" and create gate. Move lines to 8,000 and 13,500</li> <li>Run a machine calibration with calibration beads available from Luminex<sup>®</sup> Corp.</li> <li>Check stimulation conditions.</li> </ol>
Bead pattern is diffuse and missing the bead target (white oval)	<ol> <li>Precipitate buildup in system.</li> <li>Calibration is not correct.</li> <li>Incompatible buffer used to resuspend beads for Luminex<sup>®</sup> 100<sup>™</sup> analysis.</li> </ol>	<ol> <li>Drain the system, followed by a backflush, proceed with solution for air in the system.</li> <li>Run a machine calibration with calibration beads available from Luminex<sup>®</sup> Corp.</li> <li>Vacuum plate and resuspend beads in MILLIPLEX<sup>®</sup> MAP Assay Buffer.</li> </ol>
Applying vacuum to filter plate does not remove liquid from wells	<ol> <li>Wells not in use are empty.</li> <li>Particulate matter in</li> </ol>	<ol> <li>Place tape over the top of empty wells that are not in use.</li> </ol>

	lysate.	<ol> <li>Pre-filter lysate before use.</li> </ol>
Aggregation of beads during analysis	<ol> <li>Particulate matter in lysate.</li> <li>Incompatible lysis buffer.</li> <li>Beads not sonicated for step 3 of main protocol.</li> </ol>	<ol> <li>Pre-filter the lysate.</li> <li>Use recommended lysis buffer.</li> <li>Sonicate <i>resuspended</i> 1X beads prior to adding to filter plate.</li> </ol>
Liquid wicking out from 96- well filter plate	<ol> <li>Plate was not blotted on paper towel prior to adding next reagent.</li> <li>The bottom of the plate is in contact with absorbent material such as paper towel or bench paper.</li> </ol>	<ol> <li>After vacuum step, gently blot the bottom of the plate using a paper towel to remove excess liquid.</li> <li>Place the plate on a flat, non-absorbent surface during loading steps.</li> </ol>

#### **REPLACEMENT REAGENTS**

Cancer Biomarker Panel Standard 5	47-033
Cancer Biomarker Panel Standard 1	47-035
Cancer Biomarker 5, Quality Control 1 and 2	47-036
Cancer Biomarker 1, Quality Control 1 and 2	47-037
Serum and Standard Diluent (SSD)	43-050
Assay Buffer	43-051
IGF-II Activation Buffer	43-053
IGF-II Neutralization Buffer	43-054
Set of two 96-Well MultiScreen HTS Filter Plates with four Plastic Sealers	L-PLATE
Cancer Biomarker Panel Beads 5*	42-077
Cancer Biomarker Panel Beads 1**	42-078
Cancer Biomarker Panel Biotin 5	44-077
Cancer Biomarker Panel Biotin 1	44-078
Streptavidin-Phycoerythrin	45-002
10X Concentrated Wash Buffer	43-052

\*Cancer Biomarker Panel Beads 5 (#42-077): Contains MIF, Prolactin, CA-125, Leptin and Osteopontin.

\*\*Cancer Biomarker Panel Beads 1 (#42-078): Contains IGF-II.

# ORDERING INFORMATION

# To place an order:

FAX:

(636) 441-8050

Include:

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Millipore

Catalog #

- 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

Toll-Free US: (800) MILLIPORE

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<sup>®</sup> MAP products, please contact your multiplex specialist or sales representative or email our European Customer Service at <u>customerserviceEU@Millipore.com</u>.

# **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

#### **Technical Services**

For product technical assistance call or write.

Toll-Free US:	(800) MILLIPORE					
Email:	techserv dd@millipore.com					

	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 0 (Background)	Standard 4	QC-1 Control									
В	Standard 0 (Background)	Standard 4	QC-1 Control									
с	Standard 1	Standard 5	QC-2 Control									
D	Standard 1	Standard 5	QC-2 Control									
E	Standard 2	Standard 6										
F	Standard 2	Standard 6										
G	Standard 3	Standard 7										
н	Standard 3	Standard 7										