

MILLIPLEX™ MAP

MOUSE SINGLE PLEX ADIPONECTIN KIT 96 Well Plate Assay

MADPK-71K-ADPN

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INTRODUCTION

Adipocytes secrete a number of hormones called adipokines with functions that include appetite and energy balance and insulin sensitivity. One of these, adiponectin, is involved in the regulation of lipid and glucose metabolism, influencing the body's response to insulin. Also, its anti-inflammatory effects on the cellular lining of blood vessel walls may help to explain the association of high adiponectin levels with a reduced risk of heart attack.

Millipore recognizes the need to better understand the role that adiponectin plays in obesity and cardiovascular disease and is, therefore, proud to announce that the former LINCOp^{lex} Mouse Adiponectin Single Plex Panel now has the MILLIPLEX MAP optimized format. While you will immediately recognize the quality and reproducibility that you have always trusted, you will also enjoy the enhancements that we have built into MILLIPLEX MAP.

Millipore's MILLIPLEX MAP Mouse Adiponectin Single Plex Panel is to be used for the quantification of adiponectin in mouse tissue, plasma and cell/tissue culture supernatant samples. The single plex provides biomedical researchers using mouse models with quality tools for the study of obesity related disorders of metabolic disease.

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 RECONSTITUTED 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 Adipokine Standard	LMA-8071	lyophilized	1 vial
Mouse Adipokine Quality Controls 1 and 2	LMA-6071	lyophilized	2 vials
Mouse Adiponectin Detection Antibody	LMAK-1071-ADPN	5.5 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE8	5.5 mL	1 bottle
Assay Buffer Note: Contains 0.08% Sodium Azide	LE-ABGLP	30 mL	2 bottles
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	1 bottle
Set of one 96-Well Filter Plate with 2 Sealers	MX-PLATE	-----	1 plate 2 sealers
Mixing Bottle	-----	-----	1 bottle

Bead/Analyte Name	Luminex Bead Region	(20X concentration, 200 µL) Available	Cat. #
Anti-Adiponectin Bead	81	✓	MA-ADPN

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

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

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 5 μ L to 1000 μ L
2. Multichannel Pipettes capable of delivering 5 μ L to 50 μ L or 25 μ L to 200 μ L
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 with Millipore Vacuum Pump Catalog #WP6111560 or equivalent)
11. Luminex 100™ IS, 200™, or HTS by Luminex Corporation
12. Plate Stand (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.

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 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 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 μ L 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 \leq -20°C for 1 month and at \leq -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 Mixing 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 some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes. 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 background, standard curve and control wells. If samples are diluted in Assay Buffer, use the Assay Buffer as matrix.
- For serum/plasma samples, use the Serum Matrix provided in the kit.
- 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 aggregates. 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 1000xg. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- 1:5,000 dilution in Assay Buffer is recommended for serum.
- Recommended dilution Method:
 1. Add 5 μL serum to 495 μL Assay Buffer for a 1:100 dilution
 2. Add 5 μL of 1:100 diluted sample to 245 μL Assay Buffer for a final dilution of 1:5,000.

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 $\leq -20^{\circ}\text{C}$.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- 1:5,000 dilution in Assay Buffer is recommended for plasma.
- Recommended dilution Method:
 1. Add 5 μL plasma to 495 μL Assay Buffer for a 1:100 dilution
 2. Add 5 μL of 1:100 diluted sample to 245 μL Assay Buffer for a final dilution of 1:5,000.

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$.
- Avoid multiple (>2) freeze/thaw cycles.
- Tissue Culture Supernatant may require a dilution with the appropriate medium prior to assay. The sample dilution of fat tissue / cell extracts and culture media may vary.

NOTE:

- A maximum of 10 μL per well of diluted serum, plasma, or cell culture supernatant or extract can be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.

- Care must be taken when using heparin as an anticoagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

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

B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 μ L 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 portion may be stored at \leq -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 portion at 2-8°C for up to one month.

D. Preparation of Mouse Adipokine Standard

1.) Prior to use, reconstitute the Mouse Adipokine Standard with 250 μ L deionized water to give a 50,000 pg/mL concentration of standard stock. Allow the vial to sit for about 5 minutes, invert the vial several times to mix. Vortex and mix well. Transfer the standard to a microfuge tube labeled 50,000 pg/mL. The unused portions may be stored at \leq -20°C for up to one month.

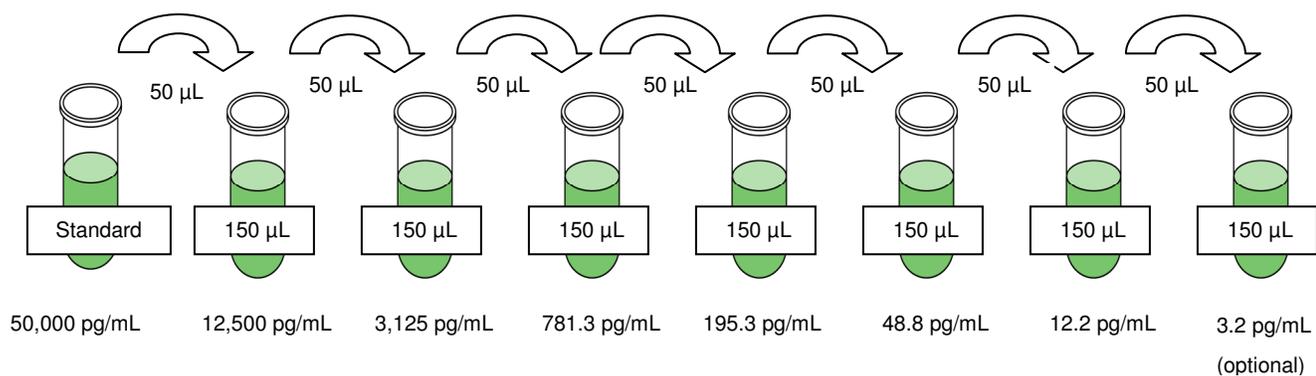
2.) Preparation of Working Standards

Label six polypropylene microfuge tubes 12,500 pg/mL, 3,125 pg/mL, 781.3 pg/mL, 195.3 pg/mL, 48.8 pg/mL, and 12.2 pg/mL. Add 150 μ L of Assay Buffer to each of the six tubes. Perform 4 times serial dilutions by adding 50 μ L of the 50,000 pg/mL standard to the 12,500 tube, mix well and transfer 50 μ L of the 12,500 standard to the 3,125 tube, mix well and transfer 50 μ L of the 3,125 standard to the 781.3 tube, mix well and transfer 50 μ L of the 781.3 standard to 195.3 tube, mix well and transfer 50 μ L of the 195.3 standard to the 48.8 tube, mix well and transfer 50 μ L of the 48.8 standard to the 12.2 tube. The 0 standard (Background) will be Assay Buffer.

Preparation of Working Standards

Standard Concentration (pg/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
50,000	-----	-----
12,500	150 μ L	50 μ L of 50,000
3,125	150 μ L	50 μ L of 12,500
781.3	150 μ L	50 μ L of 3,125
195.3	150 μ L	50 μ L of 781.3
48.8	150 μ L	50 μ L of 195.3
12.2	150 μ L	50 μ L of 48.8
3.2 (Optional)*	150 μ L	50 μ L of 12.2

* Higher sensitivity can be attained with Adiponectin using the optional standard point.



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), 12.2, 48.8, 195.3, 781.3, 3,125, 12,500, 50,000 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. Prewet the filter plate by pipetting 200 µL of Assay Buffer into each well of the Microtiter Filter 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 10 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for the 0 pg/mL standard (Background).
4. Add 10 µL of Assay Buffer to the sample wells.
5. Add 10 µL of appropriate matrix diluent to the background, standards, and control wells. When assaying tissue/cell culture supernatant samples, use similar but adiponectin-free medium. When measuring 1:5000 diluted serum or plasma sample, use Assay Buffer.
6. Add 10 µL of Sample into the appropriate wells.
7. Vortex Mixing Bottle and add 25 µL of the Mixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)
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 overnight (16-18 hours) at 2-8 °C.
9. Gently remove fluid by vacuum. (**NOTE: DO NOT INVERT PLATE.**)

Add 200 µL Assay Buffer per well



Shake 10 min, RT

Vacuum

- Add 10 µL Standard or Control to appropriate wells
- Add 10 µL Assay Buffer to background and sample wells
- Add 10 µL appropriate matrix diluent to background, standards and control wells
- Add 10 µL Samples to sample wells
- Add 25 µL Beads to each well



Incubate overnight at 2-8 °C with shaking

10. Wash plate 3 times with 200 μ L/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 μ L of Detection Antibody 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). **DO NOT VACUUM AFTER INCUBATION.**
13. Add 50 μ L Streptavidin-Phycoerythrin to each well containing the 50 μ L of Detection Antibodies. (Note: Allow the SAPE to warm to room temperature prior to addition.)
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 μ L/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 μ L of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.
18. Run plate on Luminex 100™ IS, 200™, or HTS.
19. Save and analyze the Median Fluorescent Intensity (MFI) data using a weighted 5-parameter logistic or spline curve-fitting method for calculating analyte concentrations in samples.



Vacuum and wash
3X with 200 μ L
Wash Buffer

Add 50 μ L Detection Antibody
per well



Incubate 30
minutes at RT

Do Not Vacuum

Add 50 μ L Streptavidin-
Phycoerythrin per well



Incubate for 30
minutes at RT

Vacuum and wash
3X with 200 μ L
Wash Buffer

Add 100 μ L Sheath Fluid per
well

Read on Luminex (50 μ L,
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	
Reporter Gain:	Default (low PMT)	
Time Out:	60 seconds	
Bead Set:	Adiponectin	81

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)

MinDC: Minimum Detectable Concentration 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.

Adiponectin	1.3 pg/mL
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Precision

Intra-assay precision is generated from the mean of the %CV's from multiple reportable results across different concentrations of analytes in one experiment. Inter-assay precision is generated from the mean of the %CV's from one or two reportable results for multiple concentrations of analyte across different experiments.

Intra-assay variation (%CV)	< 9.1%
Inter-assay variation (% CV)	< 4.8%

Accuracy

Spike Recovery: The data represent mean percent recovery of multiple levels of analytes spiked into several independent samples with known low analyte levels.

Adiponectin	104%
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Cross-Reactivity

The antibody pair in the panel are specific only to the desired analyte and exhibit no or negligible cross-reactivity with human adiponectin, globular domain or mouse adiponectin, or other cytokines or hormones.

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Filter plate will not vacuum	<p>Vacuum pressure is insufficient</p> <p>Samples have insoluble particles</p> <p>Sample too viscous</p>	<p>Increase vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.</p> <p>Centrifuge samples just prior to assay set-up and use supernatant.</p> <p>If high lipid concentration, after centrifugation, remove lipid layer and use supernatant.</p> <p>May need to dilute sample.</p>
Insufficient bead count	<p>Vacuum pressure too high</p> <p>Bead mix prepared incorrectly</p> <p>Samples cause interference due to particulate matter or viscosity</p> <p>Probe height not adjusted correctly</p>	<p>Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.</p> <p>Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting into the plate.</p> <p>See above. Also sample probe may need to be cleaned with alcohol flush, backflush and washes; or, if needed, probe should be removed and sonicated.</p> <p>Adjust probe to 3 alignment discs in well H6.</p>
Plate leaked	<p>Vacuum pressure too high</p> <p>Plate set directly on table or absorbent towels during incubations or reagent additions</p> <p>Insufficient blotting of filter plate bottom causing wicking</p> <p>Pipette touching plate filter during additions</p> <p>Probe height not adjusted correctly</p>	<p>Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (prewetted) plate and continue.</p> <p>Set plate on plate stand or raised edge so bottom of filter is not touching any surface.</p> <p>Blot the bottom of the filter plate well with absorbent towels after each wash step.</p> <p>Pipette to the side of well.</p> <p>Adjust probe to 3 alignment discs in well H6.</p>
Background is too high	<p>Background wells were contaminated</p> <p>Matrix used has endogenous analyte or interference</p> <p>Insufficient washes</p>	<p>Avoid cross-well contamination by using sealer appropriately and by pipeting with multichannel pipets without touching reagent in plate.</p> <p>Check matrix ingredients for crossreacting components (e.g. interleukin modified tissue culture medium).</p> <p>Increase number of washes.</p>

Beads not in region or gate	<p>Luminex not calibrated correctly or recently</p> <p>Gate settings not adjusted correctly</p> <p>Wrong bead regions in protocol template</p> <p>Incorrect sample type used</p> <p>Instrument not washed or primed</p> <p>Beads were exposed to light</p>	<p>Calibrate Luminex based on instrument manufacturer's instructions at least once a week or if temperature has changed by >3°C.</p> <p>Some Luminex instruments (e.g. Bio-Plex) require different gate settings than those described in the kit protocol. Use instrument default settings.</p> <p>Check kit protocol for correct bead regions or analyte selection.</p> <p>Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.</p> <p>Prime the Luminex 4 times to eliminate air bubbles. Wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.</p> <p>Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.</p>
Signal for whole plate is same as background	<p>Incorrect or no Detection Antibody was added</p> <p>Streptavidin-Phycoerythrin was not added</p>	<p>Add appropriate Detection Antibody and continue.</p> <p>Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been vacuumed out, sensitivity may be low.</p>
Low signal for standard curve	<p>Detection Antibody may have been vacuumed out prior to adding Streptavidin Phycoerythrin</p> <p>Incubations done at incorrect temperatures, timings or agitation</p>	<p>May need to repeat assay if desired sensitivity not achieved.</p> <p>Assay conditions need to be checked.</p>
Signals too high, standard curves are saturated	<p>Calibration target value set too high</p> <p>Plate incubation was too long with standard curve and samples</p>	<p>With some Luminex instruments (e.g. Bio-Plex) default target setting for RP1 calibrator is set at High PMT. Use low target value for calibration and reanalyze plate.</p> <p>Use shorter incubation time.</p>
Sample readings are out of range	<p>Samples contain no or below detectable levels of analyte</p> <p>Samples contain analyte concentrations higher than highest standard point</p> <p>Standard curve was saturated at higher end of curve</p>	<p>If below detectable levels, it may be possible to use higher sample volume. Check with tech support for appropriate protocol modifications.</p> <p>Samples may require dilution and reanalysis for that particular analyte.</p> <p>See above.</p>

<p>High variation in samples and/or standards</p>	<p>Multichannel pipet may not be calibrated</p> <p>Plate washing was not uniform</p> <p>Samples may have high particulate matter or other interfering substances</p> <p>Plate agitation was insufficient</p> <p>Cross-well contamination</p>	<p>Calibrate pipets.</p> <p>Confirm all reagents are vacuumed out completely in all wash steps.</p> <p>See above.</p> <p>Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without splashing.</p> <p>Check when reusing plate sealer that no reagent has touched sealer.</p> <p>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.</p>
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REPLACEMENT REAGENTS

Mouse Adipokine Standard
 Mouse Adipokine Quality Controls
 Mouse Adiponectin Detection Antibody
 Streptavidin-Phycoerythrin
 Assay Buffer
 10X Wash Buffer
 Set of two 96-Well Filter Plates with Sealers
Antibody-Immobilized Bead:

Catalog #

LMA-8071
 LMA-6071
 LMAK-1071-ADPN
 L-SAPE8
 LE-ABGLP
 L-WB
 MX-PLATE

<u>Analyte</u>	<u>Bead #</u>	<u>Cat. #</u>
Adiponectin	81	MA-ADPN

ORDERING INFORMATION

To place an order:

To assure the clarity of your custom Mouse Adiponectin 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 Standard (Background)	781.3 pg/mL Standard	QC 1 Control	Etc.								
B	0 Standard (Background)	781.3 pg/mL Standard	QC 1 Control									
C	12.2 pg/mL Standard	3,125 pg/mL Standard	QC 2 Control									
D	12.2 pg/mL Standard	3,125 pg/mL Standard	QC 2 Control									
E	48.8 pg/mL Standard	12,500 pg/mL Standard	Sample 1									
F	48.8 pg/mL Standard	12,500 pg/mL Standard	Sample 1									
G	195.3 pg/mL Standard	50,000 pg/mL Standard	Sample 2									
H	195.3 pg/mL Standard	50,000 pg/mL Standard	Sample 2									