Human Fatty Acid Oxidation (FAO) Magnetic Bead Panel 1

96-Well Plate Assay

Cat. # HFA01MAG-11K

MILLIPLEX® MAP

HUMAN FATTY ACID OXIDATION (FAO) MAGNETIC BEAD PANEL 1
96-Well Plate Assay

HFA01MAG-11K

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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION

A major focus area in current biomedical research and drug development is to study the cellular metabolic pathways that include the fatty acid oxidation (FAO) pathway. The FAO pathway, located in the mitochondria and peroxisomes, is composed of more than 25 enzymes and specific transport proteins that regulate the degradation of fatty acids (FA). It is important to monitor the FAO pathway (especially beta-oxidation pathway) and any potential cellular metabolism changes in the human tissues and cells in response to disease states, drug treatments, dietary changes or genetic mutations. For example, one of the modes of action for diabetic drug PPAR-alpha agonists is believed to induce FAO pathway enzyme expression.

The Fatty Acid Oxidation Panel 1 includes key enzymes **ACAA2, LPBE, SCHAD,** and **TFP** involved in the beta-oxidation pathway, and allows the study of these targets simultaneously in one reaction well.

To study specific metabolic pathways, it might be necessary to screen panels of specific pathway proteins that often require some level of automation and/or high throughput assays. Magnetic beads can make the process of automation and high throughput screening easier with features such as walk-away washing.
Advantages include:

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

Therefore, the **MILLIPLEX MAP** Human Fatty Acid Oxidation Panel 1 enables users to focus on the fatty acid oxidation pathway. Coupled with the Luminex® xMAP® platform in a **magnetic bead** format, users receive the advantage of ideal speed and sensitivity by allowing the quantitative multiplex detection of multiple analytes simultaneously, resulting in dramatically improved productivity.

Millipore's MILLIPLEX **MAP** Human Fatty Acid Oxidation Panel 1 is the most versatile system available for cellular metabolism research.

- MILLIPLEX **MAP** offers the ability to:
  - Choose any combination from our panel of 4 analytes to design a custom kit that better meets user needs.
- A convenient "all-in-one" box format that gives assurance that all the necessary reagents are provided to run an assay.

Millipore's MILLIPLEX **MAP** Human Fatty Acid Oxidation Panel 1 is to be used for the simultaneous detection of: **ACAA2, LPBE, SCHAD,** and **TFP**. This kit may be used for the analysis of all or any combination of the indicated analytes in cell lysate or tissue extract.

Full names and alternative names of analytes:

ACAA2: Acetyl-Coenzyme A acyltransferase 2, 3-Ketoacyl-CoA thiolase
LPBE: Peroxisomal bifunctional enzyme, PBE-1, ECHD
SCHAD: Short-chain-3-hydroxyacyl-CoA dehydrogenase, HCDH, HADH, HADHSC
TFP: Mitochondrial Trifunctional protein, HADHA / HADHB

This kit is for research purposes only.

Please read entire protocol before use.

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

PRINCIPLE

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

- Luminex® uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, 100 distinctly colored bead sets can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The microspheres are illuminated, and the internal dyes fluoresce, marking the microsphere set(s) used in a particular assay. A second illumination source 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 CONTROLS IN GLASS VIALS**. For long-term storage, freeze reconstituted controls at ≤ -70°C. Aliquot if needed. 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
HepG2 Cell Lysate Control	47-231	lyophilized	1 vial
Set of one 96-Well Plate with 2 sealers	----------	----------	1 plate 2 sealers
Assay Buffer	43-010	55 mL	1 bottle
Cell Lysis Buffer	43-045	12 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	2 bottles
FAO-1 Detection Antibodies	HFA01-1011-1	5.5 mL	1 bottle
Streptavidin-Phycoerythrin	MC-SAPE3	5.5 mL	1 bottle
Mixing Bottle	----------	----------	1 bottle

Human Fatty Acid Oxidation Panel 1 Antibody Immobilized Magnetic Beads:

Bead/Analyte Name	Luminex Magnetic Bead Region	Customizable 4 Analytes (20X concentration, 200 µL) Available	Cat. #
ACAA2	29	✓	HACAA2-MAG
LPBE	35	✓	HLPBE-MAG
SCHAD	15	✓	HSCHAD-MAG
TFP	21	✓	HTFP-MAG

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Protease inhibitors (recommend EMD Chemicals Catalog #535140 or similar product)
2. Bradford or BCA-based total protein assay (EMD Chemicals Catalog #71285 or similar product)
3. Luminex Sheath Fluid (Luminex Catalog #40-50000) or Luminex Drive Fluid (Luminex Catalog # MPXDF-4PK)

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. Aluminum Foil

7. Absorbent Pads
8. Laboratory Vortex Mixer
9. Sonicator (Branson Ultrasonic Cleaner Model # B200 or equivalent)
10. Titer Plate Shaker (Lab-Line Instruments Model #4625 or equivalent)
11. Luminex 200™, HTS, FLEXMAP 3D™, or MAGPIX® with xPONENT software by Luminex Corporation
12. Automatic Plate washer for magnetic beads (Bio-Tek ELx405, Millipore catalog #40-015 or equivalent) or Hand held Magnetic Separation Block (Millipore catalog # 40-285 or equivalent)

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

SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

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 equilibrate to room temperature (20-25°C) before use in the assay.
- Incomplete washing can adversely affect the assay outcome. All washing must be performed using the provided Wash Buffer.
- After hydration, the control must be transferred to polypropylene tubes.

- The control must be used within 1 hour of preparation. Discard any unused control except the stock which may be stored at ≤ -20°C for 1 month and at ≤ -70°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 three months.
- The plate should be read immediately after the assay is finished. If the plate cannot be read immediately, seal the plate and 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.
- When reading the assay on Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 4 alignment discs. When reading the assay on FLEXMAP 3D™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 1 alignment disc. When reading the assay on MAGPIX, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 2 alignment discs.
- Vortex all reagents well before adding to plate.

SAMPLE COLLECTION AND STORAGE

A. Considerations for Cell Stimulation.

1. Depending on the experimental design, cells can be treated with growth factors (e.g. insulin), cytokines (e.g. TNFα), or other compounds (e.g. PPAR-α agonists) before harvesting the cell lysates. The duration of stimulation in addition to the concentration of the respective factor/compound should be considered since they influence the degree of phosphorylation and protein expression of any given analyte.
2. Cellular responses to growth factors are typically improved when cells have been serum starved prior to treatment.
3. Cell lines will differ in the robustness of their signaling response for any given stimulation.

B. Preparation of cell lysates

Cell Lysis Buffer is supplied as **1X** working solution. The lysis buffer does ***NOT*** contain protease inhibitors and phosphatase inhibitor. It is recommended that protease inhibitors (EMD Chemicals, Inc., Cat. No. 535140, available separately) and phosphatase inhibitor (EMD Chemicals, Inc., Cat. No. 524629, available separately) be added immediately before use.

Suggested cell lysis protocol for adherent cells

1. After treatments, rinse cells with ice cold Phosphate Buffered Saline (PBS) and drain off PBS.
2. Add ice cold Cell Lysis Buffer with freshly added protease inhibitors to cells (0.6 mL per 150 mm dish, 0.3 mL per 100 mm dish, or 0.1 mL per well of 24-well plate).
3. Scrape adherent cells off the dish with a cell scraper. Transfer the cell suspension into a centrifuge tube and gently rock for 15-30 minutes at 4°C.
4. Centrifuge the lysate at 14,000 x g for 20 minutes at 4–8°C. Immediately transfer the cleared cell lysate (supernatant) into fresh pre-chilled micro centrifuge tubes.
5. Aliquot and store the lysate at ≤ -70°C. The lysate should be stable for several months.
6. It is recommended that the lysate be diluted at least 1:10 in PBS for determining the protein concentration with Coomassie Bradford assays or 1:4 for BCA assays.

Suggested cell lysis protocol for non-adherent cells

1. Pellet the cells by centrifugation (500 – 1000 x g) in a tabletop centrifuge for 5 minutes.
2. Wash the cells in ice cold PBS.
3. Add ice cold Cell Lysis Buffer containing freshly prepared protease inhibitors to cells (1 mL per 1 x 10^7 cells).
4. Gently rock the lysate for 15-30 minutes at 4°C.
5. Centrifuge the lysate at 14,000 x g for 20 minutes at 4–8°C. Immediately transfer the cleared cell lysate (supernatant) into fresh pre-chilled micro centrifuge tubes
6. Aliquot and store the lysate at ≤ -70°C. The lysate should be stable for several months.
7. It is recommended that the lysate be diluted at least 1:10 in PBS for determining the protein concentration with Coomassie Bradford assays or 1:4 for BCA assays.

Cell lysis protocol for cells in sterile 96-well tissue culture plates

Adherent or non-adherent cells seeded or grown in sterile 96-well tissue culture grade plates (see supplemental protocols) can be washed, treated, and lysed in the same plate, but need to be filtered in a separate 96-well filter plate. Wash the cells by centrifugation in a microplate carrier for 2 minutes at 500 x g.

1. Remove the supernatant via aspiration and add 100 µL of ice cold PBS.
2. Centrifuge and remove supernatant via aspiration.
3. Add 30-50 µL/well of ice cold Cell Lysis Buffer with freshly added protease inhibitors.
4. Place the plate on an orbital shaker (600 – 800 rpm) for 10-15 minutes at 4°C.
5. Transfer the lysate to a 96-well filter plate that has been pre-wetted with 1X Cell Lysis Buffer.
6. Place a low protein binding, 96-well round bottom or V-bottom plate underneath the filter plate.
7. Centrifuge the plates in a microplate carrier for 5 minutes at 500 x g.
8. Store the filtered lysate at ≤ -70°C until ready for use.
9. It is recommended that the lysate be diluted at least 1:10 in PBS for determining the protein concentration with Coomassie Bradford assays or 1:4 for BCA assays.

C. Preparation of tissue extracts

Cell Lysis Buffer is supplied as **1X** working solution. The lysis buffer does ***NOT*** contain protease inhibitors and phosphatase inhibitor. It is recommended that protease inhibitors (EMD Chemicals, Inc., Cat. No. 535140, available separately) and phosphatase inhibitor (EMD Chemicals, Inc., Cat. No. 524629, available separately) be added immediately before use.

Suggested protocol for tissue extracts preparation

1. Weigh out the appropriate amount of tissue. Wash the sample tissue twice with ice-cold Phosphate Buffered Saline (PBS).
2. Mince the tissue with a scalpel and place in pre-chilled 2 mL Dounce homogenizer. Add **up to** 2.0 mL ice cold Cell Lysis Buffer with freshly added protease inhibitors to the tissue in the homogenizer.
3. To rupture the cells, perform 20–50 Dounce strokes, use pestle A (large clearance) for the initial strokes, then use pestle B (small clearance) for the remaining strokes. Tissue homogenate can be stored at ≤ –70°C in aliquots at this point.
4. To prepare tissue extracts, estimate the volume of the tissue homogenate and add 4 extra volumes of ice cold Cell Lysis Buffer with freshly added protease inhibitors.
5. Incubate on ice for 30 minutes with occasional vortexing. Centrifuge the tissue extracts at 14,000 x g for 20 minutes at 4–8°C. Immediately aliquot the cleared extracts into fresh microcentrifuge tubes and discard the pellet.
6. Store the tissue extract aliquot at ≤ -70°C. The tissue extract should be stable for several months.
7. It is recommended that the tissue extract be diluted at least 1:10 with PBS for determining the protein concentration with Coomassie Bradford assays or 1:4 for BCA assays.

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

Sonicate each individual antibody-bead vial for 30 seconds then vortex for 1 minute. Add 150 µL from each 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. (Note: Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit.)

Example: When using 4 antibody-immobilized beads, add 150 µL from each of the 4 bead vials to the Mixing Bottle. Then add 2.4 mL Assay Buffer.

B. 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.

C. Preparation of lyophilized HepG2 Lysate Control (Catalog #47-231)

HepG2 Lysate Control (#47-231) is provided as a lyophilized stock of cell lysate prepared from unstimulated HepG2 cells and is used as a positive control.

1. Reconstitute each of the lyophilized cell lysates in 100 µL of ultrapure water, for each vial this will yield 100 µL of lysate at a total protein concentration of 2 mg/mL. If desired, unused lysate may be stored in polypropylene vials at ≤ -70°C for up to one month.
2. Gently vortex and incubate the reconstituted lysates for 5 min at RT (store on ice).
3. Mix 10 µL of the 2 mg/mL cell lysate with 90 µL of Assay Buffer in a micro centrifuge tube. The prepared cell lysate control is now ready for use.

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 equilibrate to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Blanks, Controls, 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 samples in duplicate.
- If using a filter plate, set the filter plate on a plate holder at all times during reagent dispensing and incubation steps to ensure that bottom of the plate does not touch any surface.

1. Dilute lysate samples **at least** 1:1 in Assay Buffer. The suggested working range of protein concentration for the assay is **1 to 5** µg of total protein/well (25 µL/well at 40 to 200 µg/mL).

   No additional dilution is necessary for the prepared Lysate Controls.

2. Add 200 µL of Assay Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).

3. Decant Assay Buffer and remove the residual amount from all wells by inverting the plate and tapping it onto absorbent towels several times.

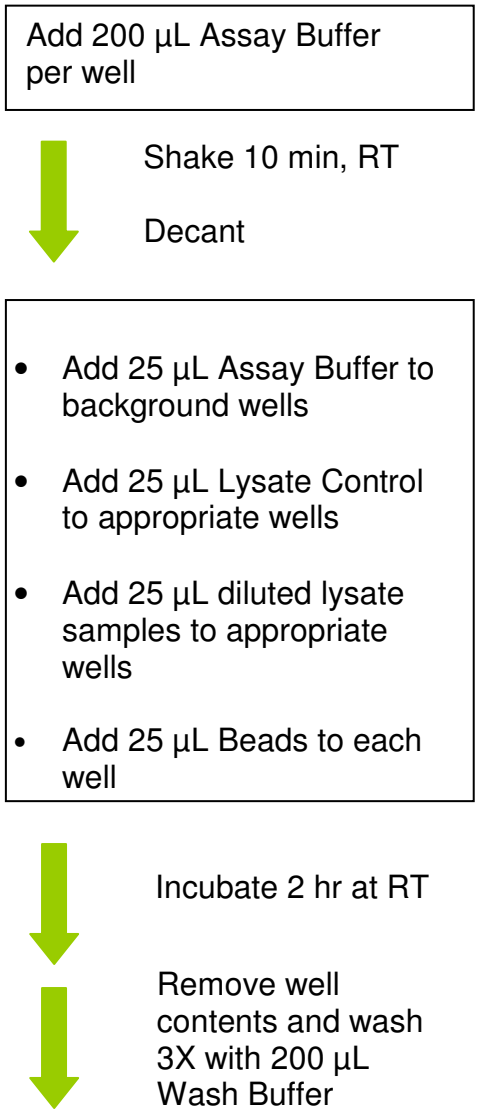
4. Add 25 µL of Assay Buffer to the background wells.

5. Add 25 µL of the Lysate Control into the appropriate wells.

6. Add 25 µL of diluted lysate 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 the plate with foil and incubate with agitation on a plate shaker 2 hours at room tem



9. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.

10. Add 50 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition).

11. Seal, cover with lid and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C).

12. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.

13. Add 50 µL Streptavidin-Phycoerythrin to each well.

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 well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.

16. Add 100 µL of Sheath Fluid (or Drive Fluid if using MAGPIX®) to all wells. Resuspend the beads on a plate shaker for 5 minutes.

17. Run plate on Luminex 200™, HTS, FLEXMAP 3D™ or MAGPIX® with xPONENT software.

18. Save and analyze the Median Fluorescence Intensity (MFI) data. (Note: make sure all lysate samples in comparison are diluted by the same factor.)

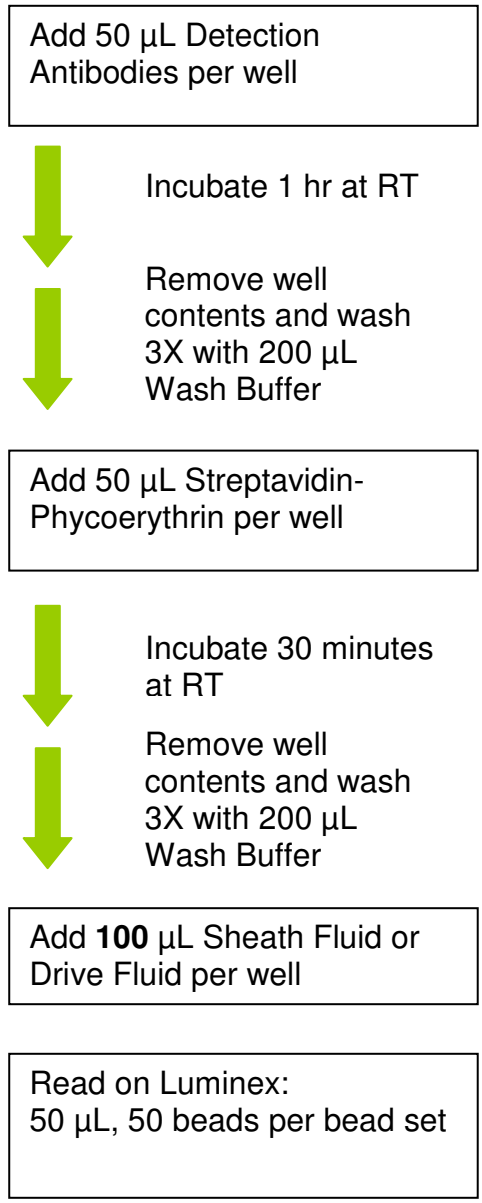


PLATE WASHING

1.) Solid Plate

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

A.) For hand-held magnet, rest plate on magnet for 60 seconds to allow complete settling of magnetic beads. Remove well contents by gently decanting the plate in an appropriate waste receptacle and gently tapping on absorbent pads to remove residual liquid. Wash plate with 200 µL of Wash Buffer by removing plate from magnet, adding Wash Buffer, shaking for 30 seconds, reattaching to magnet, letting beads settle for 60 seconds and removing well contents as previously described after each wash. Repeat wash steps as recommended in Assay Procedure.

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

2.) Filter Plate (Millipore Cat# MX-PLATE)

If using a filter plate, use a vacuum filtration manifold to remove well contents. Wash plate with 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration after each wash. Repeat wash steps as recommended in the Assay Procedure.

EQUIPMENT SETTINGS

Bio-Tek ELx405:

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

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

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

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

   Dispense:
   1. Dispense volume: 200 µL/well

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

Aspiration:

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

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

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

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

EQUIPMENT SETTINGS (continued)

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

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

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

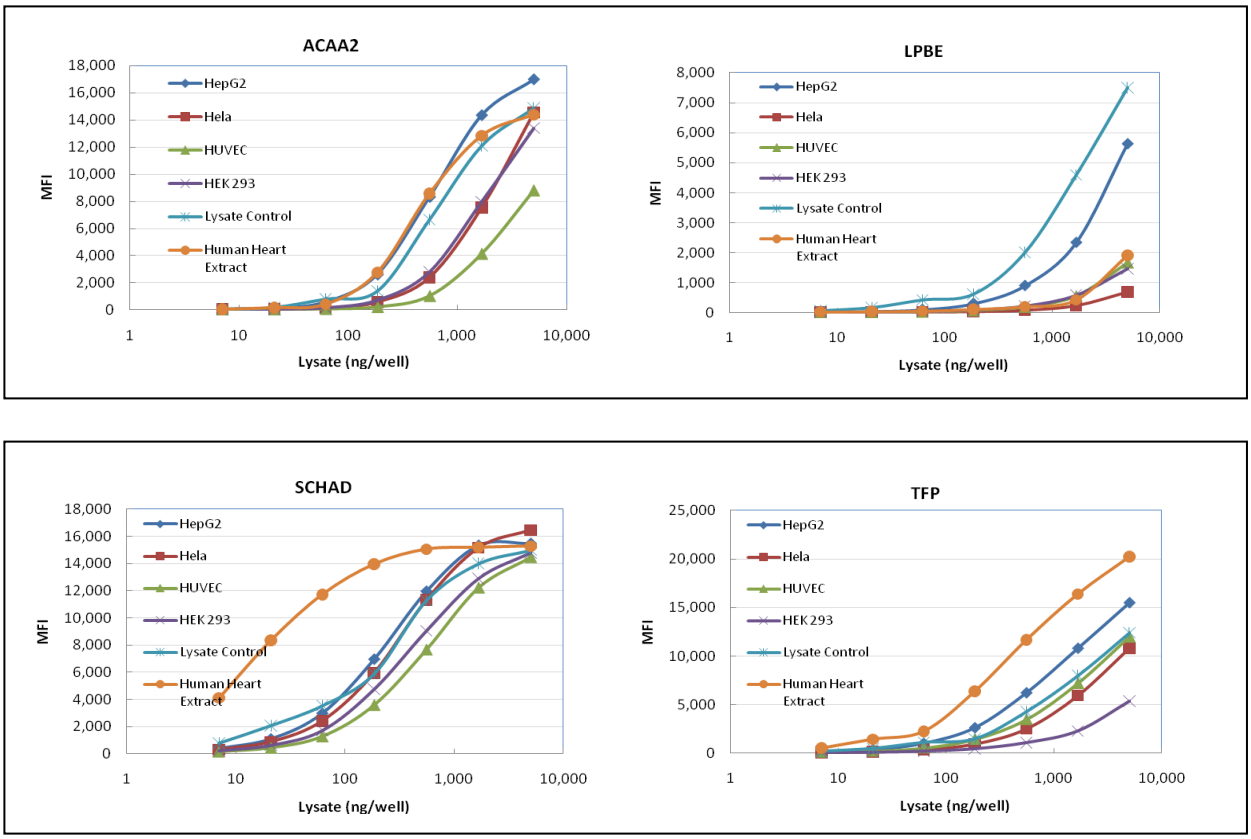
NOTE: These assays cannot be run on any instruments using Luminex IS 2.3 or Luminex 1.7 software.

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

Events:	50, per bead	
Sample Size:	50 µL	
Gate Settings:	8,000 to 15,000	
Reporter Gain:	Default (low PMT)	
Time Out:	60 seconds	
Bead Set:	Customizable 4-Plex Beads	
	SCHAD	15
	TFP	21
	ACAA2	29
	LPBE	35

ASSAY CHARACTERISTICS

Representative Data



Multiplex analysis of human cell lysates and tissue extract with Human Fatty Acid Oxidation (FAO) Magnetic Bead Panel 1. HepG2, HeLa, HUVEC cell lysates, human heart extract and HepG2 Lysate Control were prepared according to the procedures described in the protocol. The lysates were serially diluted with Assay Buffer and analyzed with Human Fatty Acid Oxidation (FAO) Magnetic Bead Panel 1 according to the assay protocol. The Median Fluorescence Intensity (MFI) was measured with the Luminex® system.

Cross-Reactivity

There was no or negligible cross-reactivity between the antibodies for an analyte and any of the other analytes within this panel.

Precision

Intra-assay precision is generated from the mean of the %CVs from 8 reportable results across two different concentrations of analytes in a single assay. Inter-assay precision is generated from the mean of the %CVs from 48 reportable results across two different concentrations of analytes from 6 different assays.

Analyte	Intra-Assay CV	Inter-Assay CV
ACAA2	2.8%	4.5%
LPBE	3.4%	5.6%
SCHAD	1.3%	3.5%
TFP	2.3%	5.4%

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Insufficient Bead Count	Plate Washer aspirate height set too low	Adjust aspiration height according to manufacturers' instructions.
	Bead mix prepared inappropriately	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting this into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with Alcohol flush, Back flush and washes; or if needed probe should be removed and sonicated.
	Probe height not adjusted correctly	When reading the assay on Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 4 alignment discs. When reading the assay on FLEXMAP 3D™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 1 alignment disc. When reading the assay on MAGPIX®, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 2 alignment discs.
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.
	Insufficient washes	Increase number of washes.
Beads not in region or gate	Luminex instrument not calibrated correctly or recently	Calibrate Luminex instrument based on Instrument Manufacturer's instructions, at least once a week or if temperature has changed by >3°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 get rid of air bubbles, wash 4 times with sheath fluid or water if there is any residual 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.
Low signal for Lysate Control	Incubations done at inappropriate temperatures, timings or agitation	Assay conditions need to be checked.

Sample signals too high and saturated	Calibration target value set too high	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.
	Plate incubation was too long with Lysate Control and samples	Use shorter incubation time.
	Samples contain analyte concentrations higher than the assay dynamic range.	Samples may require dilution and re-analysis for just that particular analyte.
Sample signals too low	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.
High Variation in samples and/or standards	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm all reagents are removed completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without causing splashing.
	Cross well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.
FOR FILTER PLATES ONLY		
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2 mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay setup and use supernatant.
	High lipid concentration	After centrifugation, remove lipid layer and use supernatant.
Plate leaked	Vacuum Pressure too high	Adjust vacuum pressure such that 0.2 mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (blocked) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate holder or raised edge so bottom of filter is not touching any surface.
	Insufficient blotting of filter plate bottom causing wicking	Blot the bottom of the filter plate well with absorbent towels after each wash step.
	Pipette touching plate filter during additions	Pipette to the side of plate.

	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
	Sample too viscous	May need to dilute sample.

REPLACEMENT REAGENTS	**Catalog #**
HepG2 Cell Lysate Control	47-231
Human Fatty Acid Oxidation Panel 1 Detection Antibodies	HFA01-1011-1
Assay Buffer	43-010
Cell Lysis Buffer	43-045
Streptavidin-Phycoerythrin	MC-SAPE3
Set of two 96-Well plate with Sealers	MAG-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Magnetic Beads

Analyte	Bead #	Catalog #
ACAA2	29	HACAA2-MAG
LPBE	35	HLPBE-MAG
SCHAD	15	HSCHAD-MAG
TFP	21	HTFP-MAG

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WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Background	Sample 3										
B	Background	Sample 3										
C	Lysate Control	Etc.										
D	Lysate Control											
E	Sample 1											
F	Sample 1											
G	Sample 2											
H	Sample 2											