MILLIPLEX® MAP

Human Brain-Derived Protein Panel Protocol
96 Well Plate Assay

HBDP-33K

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FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION

Brain-derived proteins, such as hypothalamus neuropeptides and pituitary hormones, play very important roles in the regulation of various functions including metabolism, growth, and reproduction. Accurate measurement of these proteins to understand their new biological functions and molecular mechanisms of the functions are crucial. Traditional laboratory methods, such as RIA and ELISA are not able to measure multiple proteins with a small sample volume.

Millipore recognizes the integral role that these proteins play and is therefore proud to announce the new Milliplex MAP multiplex Human Brain-derived Protein Panel for the simultaneous measurement of the following analytes in any combination: AGRP, FSH, BDNF, LH, TSH, PRL, GH, ACTH and CNTF. This multiplex assay can analyze all 9 proteins simultaneously and uses a small sample volume 25µL.

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.

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

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- Once the standards and controls have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE RECONSITUTED STANDARDS OR CONTROLS IN GLASS VIALS**. For long-term storage, freeze reconstituted standards and controls at ≤ -20°C. Avoid multiple (>2) freeze thaw cycles.
- **DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibody, and Streptavidin-Phycoerythrin**.

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8 °C

REAGENTS SUPPLIED	Catalog Number	Volume	Quantity
Human Brain Derived/Pituitary Standard	HBDP-8033	lyophilized	1 vial
Human Brain Derived/ Pituitary Controls 1 and 2	HBDP-6033	lyophilized	2 vials
Set of one 96-Well filter Plate with 2 sealers	MX-PLATE	----------	1 plate 2 sealers
Assay Buffer	L-AB	30 mL	1 bottle
Serum Matrix	LHPT-SM	1 mL	1 bottle
Bead Diluent	LBD	3.5 mL	1 vial
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	1 bottle
Human Brain-Derived Protein Panel Detection Antibodies	HBDP-1033	5.5 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE	5.5 mL	1 bottle
Mixing Bottle	----------	----------	1 bottle

Human Brain-Derived Protein Antibody Immobilized Beads:

Bead/Analyte Name	Luminex Bead Region	Customizable 9 Analytes (20X concentration, 200µL) Available	Cat. #
Anti-AGRP	1	✓	HAGRP
Anti-FSH	2	✓	HPT-FSH
Anti-BDNF	9	✓	RBDNF
Anti-LH	13	✓	HPT-LH
Anti-TSH	24	✓	HPT-TSH
Anti-Prolactin	27	✓	HPT-PRL
Anti-GH	43	✓	HPT-GH
Anti-ACTH	48	✓	HPT-ACTH
Anti-CNTF	52	✓	HCNTF

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. Laboratory Vortex Mixer
7. Sonicator (Branson Ultrasonic Cleaner Model # B200 or equivalent)
8. Titer Plate Shaker (Lab-Line Instruments, Model #4625, or equivalent)
9. Luminex 100™, IS, 200™, or HTS by Luminex Corporation
10. Vacuum Filtration Unit (Millipore Vacuum Manifold Catalog #MSVMHTS00, or equivalent. Millipore Vacuum Pump Catalog #WP6111560 or equivalent.)
11. 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.

- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- 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 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 blank, standard curve and controls. If samples are diluted in assay buffer, use the assay buffer as matrix.
- For serum / plasma samples, use the assay buffer provided in the kit to dilute samples (1:3) and Serum Matrix (1:3).
- 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. 25 µL per well of 1:3 serum or plasma should be used. The sample dilution of tissue/cell extracts and culture media may vary.

Note: Dilute serum or plasma samples 1:3 with Assay Buffer. Make dilution with 25 µL serum or plasma sample to 50 µL of Assay Buffer and mix well.

B. 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. Use Assay Buffer to dilute serum samples prior to assay.

C. Preparation of Plasma Samples:

Centrifuge for 10 minutes at 1000 xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20ºC. If measuring ACTH, store plasma samples at -70 ºC. Avoid multiple (>2) freeze/thaw cycles. It is recommended to centrifuge plasma samples again at 3000 xg for five minutes prior to assay set up. Use Assay Buffer to dilute serum samples prior to assay.

D. 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.

Note:

- 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 each antibody-bead vial for 30 seconds; 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 Bead Diluent. Vortex the mixed beads well. Unused portions may be stored at 2-8°C for up to one month.

Example 1: when using 9 antibody-immobilized beads, add 150 µL from each of the 9 bead sets to the Mixing Bottle. Then add 1.65 mL Bead Diluent

Example 2: when using 3 antibody-immobilized beads, add 150 µL from each of the 3 bead sets to the Mixing Bottle. Then add 2.55 mL Bead Diluent.

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 Serum Matrix

This step is required for serum or plasma samples only.
Add 1.0 mL deionized water and 2.0 mL of Assay Buffer to the bottle containing lyophilized Serum Matrix. The final matrix dilution is 1:3. Mix well. Allow at least 10 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at ≤ -20°C for up to one month.

E. Preparation of Human Brain-Derived Protein Standard

1.) Prior to use, reconstitute the Human Brain-Derived Protein Standard with 250 µL Deionized Water. 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 Standard 7.

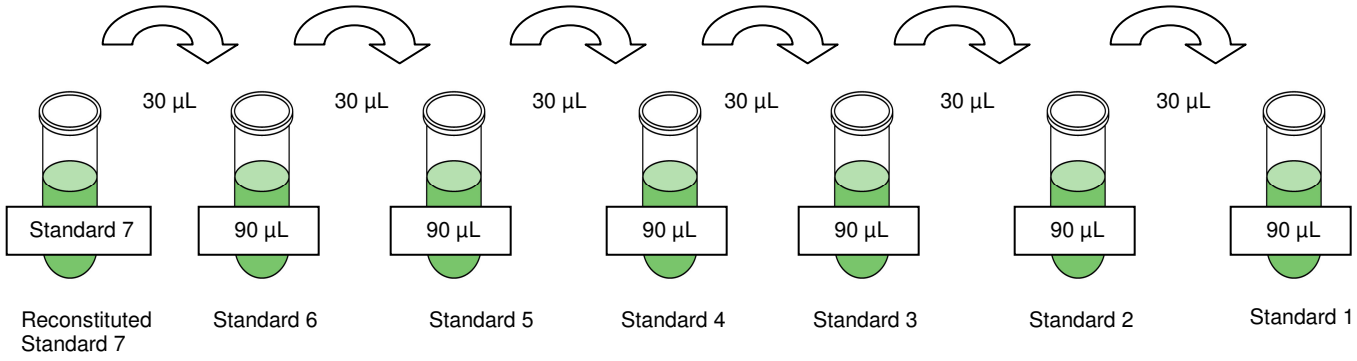
2.) Preparation of Working Standards

Label 6 polypropylene microfuge tubes “Standard 6,” “Standard 5,” “Standard 4,” “Standard 3,” “Standard 2,” and “Standard 1.” Add 90 µL Assay Buffer to each of the six tubes. Perform 4 times serial dilutions by adding 30 µL of the “Standard 7” to the “Standard 6” tube, mix well and transfer 30 µL of the “Standard 6” to the “Standard 5” tube, mix well and transfer 30 µL of the “Standard 5” to “Standard 4” tube, mix well and transfer 30 µL of the “Standard 4” to the “Standard 3”, mix well and transfer 30 µL of the “Standard 3” to the “Standard 2” tube, mix well and transfer 30 µL of the “Standard 2” to the “Standard 1”. The 0 Standard (background) will be Assay Buffer.

Preparation of Working Standards

Standard	Volume of Deionized Water to Add	Volume of Standard to Add
Standard 7	250 µL	0

Standard	Volume of Assay Buffer to Add	Volume of Standard to Add
Standard 6	90 µL	30 µL of Standard 7
Standard 5	90 µL	30 µL of Standard 6
Standard 4	90 µL	30 µL of Standard 5
Standard 3	90 µL	30 µL of Standard 4
Standard 2	90 µL	30 µL of Standard 3
Standard 1	90 µL	30 µL of Standard 2



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

Standard Tube #	AGRP (pg/mL)	FSH (mIU/mL)	BDNF, GH (pg/mL)	LH (mIU/mL)	TSH (µIU/mL)	Prolactin (pg/mL)	ACTH (pg/mL)	CNTF (pg/mL)
1	2.4	0.024	12	0.049	0.024	24	3.0	122
2	10	0.098	49	0.195	0.098	98	12	488
3	39	0.39	195	0.781	0.391	390	49	1,953
4	156	1.56	781	3.125	1.56	1,563	195	7,813
5	625	6.25	3,125	12.5	6.25	6,250	781	31,250
6	2,500	25	12,500	50	25	25,000	3,125	125,000
7	10,000	100	50,000	200	100	100,000	12,500	500,000

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), Std 1, Std 2, Std 3, Std 4, Std 5, Std 6, and Std 7], Controls 1 and 2, and samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.
- Set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

1. Prewet the filter plate by pipetting 200 µL of Assay Buffer into each well of the Microtiter Filter Plate. Seal and shake 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 Assay Buffer to the Background wells. Add 25 µL of each Standard or Control into the appropriate wells.
4. Add 25 µl Assay buffer in Sample wells.
5. Add 25 µL of diluted samples to the Sample wells.
6. Add 25 µL of diluted Serum Matrix (when measuring serum or plasma samples) or appropriate culture media (when measuring culture samples) in Background, Standards, and Quality Control wells.
7. Vortex Bead Bottle and add 25 µL of the prepared Beads to each well. (Note: during addition of the Beads, shake beads 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 for overnight incubation at 4°C (16-18 hours).
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 between each wash.

Add 200µL Assay Buffer per well

Shake 10 min, RT

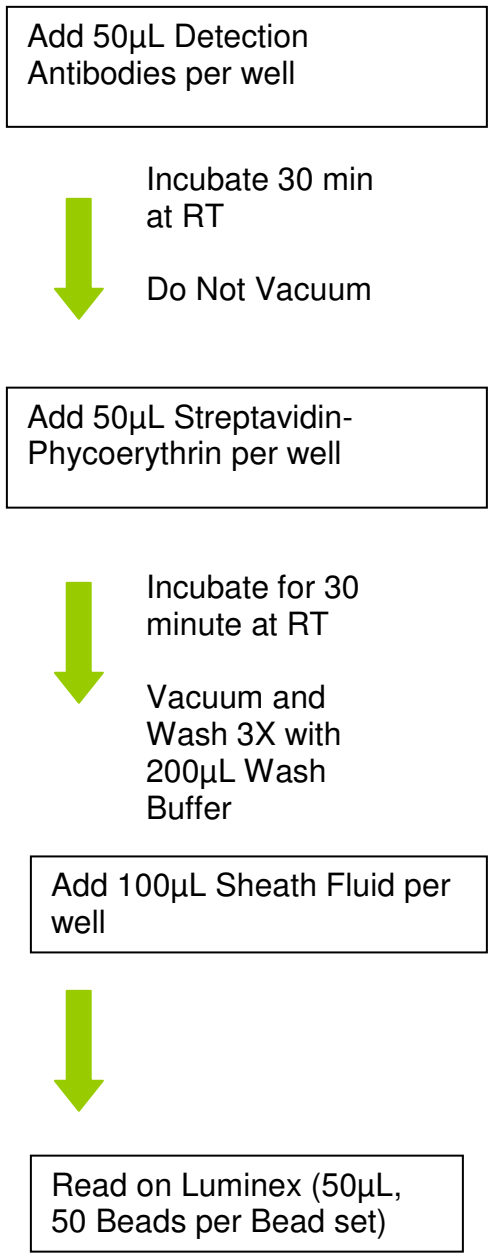
Decant

- Add 25µL Assay Buffer to background wells and 25µL Standard, and 25µL Controls to Standard and Control wells, respectively.
- Add 25µL Assay Buffer to Sample wells.
- Add 25µL diluted samples to Sample Wells.
- Add 25µL diluted Serum Matrix (or appropriate media) to Background, Standard and Quality Controls.
- Add 25 µL Beads to each well

Incubate overnight at 4°C with agitation on a plate shaker.

Vacuum and Wash 3X with 200µL Wash Buffer

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) **DO NOT WASH AFTER INCUBATION.**
13. Add 50 µL Streptavidin-Phycoerythrin to each well containing the 50 µL of Detection Antibodies.
14. Seal, cover with lid and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
15. Gently remove all contents by vacuum.
16. Wash plate 3 times with 200 µL/well Wash Buffer, removing Wash Buffer vacuum between each wash.
17. Add 100 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.
18. Run plate on Luminex$^{100, 200, HTS}$.
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.



EQUIPMENT SETTINGS

These specifications are for the Luminex^{100}, ^{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:	9-Plex Beads	
	AGRP	1
	FSH	2
	BDNF	9
	LH	13
	TSH	24
	Prolactin	27
	GH	43
	ACTH	48
	CNTF	52

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)

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 = 6 assays)
	MinDc
AGRP (pg/mL)	1.0
FSH (mIU/mL)	0.01
BDNF (pg/mL)	2.0
LH (mIU/mL)	0.01
TSH (µIU/mL)	0.01
Prolactin (pg/mL)	5.0
GH (pg/mL)	1.0
ACTH (pg/mL)	1.0
CNTF (pg/mL)	89

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentration of analytes 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 analytes in different experiments.

Analyte	Intra-Assay (%CV)	Inter-Assay (%CV)
AGRP	< 10	< 10
FSH	< 10	< 10
BDNF	< 10	< 10
LH	< 10	< 10
TSH	< 10	< 10
Prolactin	< 10	< 10
GH	< 10	< 10
ACTH	< 10	< 10
CNTF	< 10	< 10

Accuracy

Spike Recovery: The data represents mean recovery of 3 levels of spiked standards in serum matrix samples.

Analyte	Spike Recovery (% Recovery)
AGRP	91
FSH	91
BDNF	89
LH	92
TSH	88
Prolactin	91
GH	89
ACTH	87
CNTF	95

Cross-Reactivity

The antibody pairs in the panel are specific only to the desired analyte and exhibit no or negligible cross-reactivity with other analytes in the panel.

TROUBLESHOOTING GUIDE

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

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

High Variation in samples and/or standards	Multichannel pipet may not be calibrated	Calibrate pipets
	Plate washing was not uniform	Confirm all reagents are vacuumed out completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without causing splashing
	Cross well contamination	Check when reusing plate sealer that no reagent has touched sealer. Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.

REPLACEMENT REAGENTS	**CATALOG #**
Human Brain-Derived/Pituitary Standard	HBDP-8033
Human Brain-Derived/ Pituitary Quality Controls	HBDP-6033
Human Brain-Derived Protein Panel Detection Antibodies	HBDP-1033
Serum Matrix	LHPT-SM
Bead Diluent	LBD
Assay Buffer	L-AB
Streptavidin-Phycoerythrin	L-SAPE
Set of two 96-Well plates with sealers).	MX-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Beads

Analyte	Bead #	Cat. #
Anti-AGRP	1	HAGRP
Anti-FSH	2	HPT-FSH
Anti-BDNF	9	RBDNF
Anti-LH	13	HPT-LH
Anti-TSH	24	HPT-TSH
Anti-Prolactin	27	HPT-PRL
Anti-GH	43	HPT-GH
Anti-ACTH	48	HPT-ACTH
Anti-CNTF	52	HCNTF

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
- Selection of MILLIPLEX® Analytes

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)	Standard 4	QC-1 Control	Etc.								
B	0 Standard (Background)	Standard 4	QC-1 Control									
C	Standard 1	Standard 5	QC-2 Control									
D	Standard 1	Standard 5	QC-2 Control									
E	Standard 2	Standard 6	Sample 1									
F	Standard 2	Standard 6	Sample 1									
G	Standard 3	Standard 7	Sample 2									
H	Standard 3	Standard 7	Sample 2									