

**Rat Pituitary Magnetic  
Bead Panel**

**96 Well Plate Assay**

**Cat. # RPTMAG-86K**

# MILLIPLEX<sup>®</sup> MAP

## RAT PITUITARY MAGNETIC BEAD PANEL 96 Well Plate Assay

#RPTMAG-86K

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

By purchasing this product, which contains fluorescently labeled microsphere beads authorized by Luminex Corporation ("Luminex"), you, the customer, acquire the right under Luminex's patent rights, if any, to use this product or any portion of this product, including without limitation the microsphere beads contained herein, only with Luminex's laser based fluorescent analytical test instrumentation marketed under the name of Luminex 100<sup>™</sup> IS, 200<sup>™</sup>, HTS, FLEXMAP 3D<sup>™</sup>, MAGPIX<sup>®</sup>.

## INTRODUCTION

Called the “master gland” because it controls many other endocrine glands, the pituitary gland secretes a number of key hormones that play important roles in the regulation of metabolism, growth, and reproduction. For example, these hormones are responsible for the stimulation of the adrenal gland (ACTH), thyroid gland (TSH), ovaries and testes (FSH, LH) and breast milk production (prolactin), as well as blood pressure and the amount of water excreted by the kidneys. In turn, the pituitary gland is connected to and controlled by the hypothalamus, which determines the amount of stimulation needed by the pituitary’s target glands by detection of their hormone levels. Brain-Derived Neurotrophic Factor (BDNF) is a member of NGF family neurotrophic factors which are required for differentiation and survival of specific neuronal sub-populations in the nervous systems.

To identify specific pituitary hormones it might be necessary to screen panels of pituitary hormones, often requiring some level of automation and/or high throughput. Magnetic Beads can make the process of automation and high throughput screening easier with features such as walk-away washing. Advantages even outside automation include:

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

EMD Millipore recognizes the integral role that the pituitary hormones play in metabolism, growth, and reproduction. Therefore, the **MILLIPLEX<sup>®</sup> MAP** Rat Pituitary Panel enables you to explore the study of reproduction, growth, metabolic homeostasis, and pituitary-related diseases such as acromegaly, growth hormone deficiency, diabetes insipidus and pituitary tumors. Coupled with the Luminex xMAP<sup>®</sup> platform in a **magnetic bead** format, you receive the advantage of ideal speed and sensitivity, allowing quantitative multiplex detection of dozens of analytes simultaneously, which can dramatically improve productivity.

EMD Millipore’s MILLIPLEX MAP Rat Pituitary Panel is the most versatile system available for pituitary hormone research.

- MILLIPLEX MAP offers you the ability to:
  - Choose any combination of analytes from our panel of 7 analytes to design a custom kit that better meets your needs.
- A convenient “all-in-one” box format gives you the assurance that you will have all the necessary reagents you need to run your assay.

EMD Millipore’s MILLIPLEX MAP Rat Pituitary Panel kit is to be used for the simultaneous quantification of the following 7 rat pituitary hormones: Growth Hormone (GH), Thyroid-Stimulating Hormone (TSH), Adrenocorticotrophic Hormone (ACTH), Luteinizing Hormone (LH), Prolactin, Follicle-Stimulating Hormone (FSH), and Brain-Derived Neurotrophic Factor (BDNF).

This kit may be used for the analysis of all or any combination of the above pituitary hormones in rat serum or plasma samples, rat tissue extract, or cell/tissue culture supernatant samples.

***For Research Use Only. Not for Use in Diagnostic Procedures.***

***Please read entire protocol before use.***

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

## PRINCIPLE

MILLIPIX MAP are 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 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 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  $\leq -20^{\circ}\text{C}$ . 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
Rat Pituitary Standard	LRPT-8086	lyophilized	1 vial
Rat Pituitary Quality Controls 1 and 2	LRPT-6086	lyophilized	2 vials
Rat Pituitary Detection Antibodies	LRPT-1086	5.5 mL	1 bottle
Serum Matrix Note: Contains 0.08% Sodium Azide	LRPT-SM	lyophilized	1 vial (required for serum and plasma samples only)
Bead Diluent	LHE-BD	3.0 mL	1 bottle
Assay Buffer	LE-ABGLP	30 mL	2 bottles
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	2 bottles
Streptavidin-Phycoerythrin	L-SAPE	5.5 mL	1 bottle
Set of one 96-Well Plate with 2 sealers	-----	-----	1 plate 2 sealers
Mixing Bottle	-----	-----	1 bottle

### Rat Pituitary Antibody-Immobilized Beads:

Bead/Analyte Name	Luminex Magnetic Bead Region	Customizable 7 Analytes (20X concentration, 200µL)	
		Available	Cat. #
Anti-ACTH Bead	13	✓	RACTH-MAG
Anti-BDNF Bead	15	✓	RBDNF-MAG
Anti-FSH Bead	26	✓	RFSH-MAG
Anti-GH Bead	28	✓	RGH-MAG
Anti-LH Bead	43	✓	RLH-MAG
Anti-Prolactin Bead	45	✓	RPRL-MAG
Anti-TSH Bead	62	✓	RTSH-MAG

## **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  $\mu\text{L}$  to 1000  $\mu\text{L}$
2. Multichannel Pipettes capable of delivering 5  $\mu\text{L}$  to 50  $\mu\text{L}$  or 25  $\mu\text{L}$  to 200  $\mu\text{L}$
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 or FLEXMAP 3D or MAGPIX® with xPONENT software by Luminex Corporation
12. Automatic Plate washer for magnetic beads (Bio-Tek ELx405, EMD Millipore catalog #40-015) ) or Hand-held Magnetic Separation Block (EMD Millipore Catalog #40-285 or equivalent)

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

## TECHNICAL GUIDELINES (continued)

- 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.
- 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  $\leq -20^{\circ}\text{C}$  for 1 month and at  $\leq -80^{\circ}\text{C}$  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.
- 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.
- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in background, standard 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 and no 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.
- Serum samples should be diluted 1:3 in the diluted Serum Matrix provided in the kit (e.g. add 25  $\mu\text{L}$  of serum to 50  $\mu\text{L}$  diluted Serum Matrix and mix well).

### B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anticoagulant 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.
- Plasma samples should be diluted 1:3 in the diluted Serum Matrix provided in the kit (e.g. add 25  $\mu\text{L}$  of plasma to 50  $\mu\text{L}$  diluted Serum Matrix and mix well).

### 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 an appropriate control medium prior to assay.

### NOTE:

- A maximum of 25  $\mu\text{L}$  per well of 1:3 diluted serum or plasma can be used (dilute the serum or plasma with 1:3 diluted Serum Matrix). Tissue culture or other media may also 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 each individual antibody-bead vial for 30 seconds; vortex for 1 minute. Add 150  $\mu$ L 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 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 1: When using 1 antibody-immobilized bead, add 150  $\mu$ L from the bead vial to the Mixing Bottle. Then add 2.85 mL Bead Diluent.

Example 2: When using 7 antibody-immobilized beads, add 150  $\mu$ L from the bead vial to the Mixing Bottle. Then add 1.95 mL Bead Diluent.

### B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250  $\mu$ L deionized water. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes. 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 Serum Matrix

**This step is required for serum or plasma samples only.**

Add 2.0 mL deionized water to the bottle containing lyophilized Serum Matrix. Mix well. Allow the bottle to sit for 5-10 minutes for complete reconstitution. Then add 4.0 mL of Assay Buffer to the bottle and mix well to make the 1:3 diluted Serum Matrix used throughout the assay. This Serum Matrix may be frozen ( $\leq$  -20°C) and re-used twice.

## PREPARATION OF REAGENTS FOR IMMUNOASSAY (continued)

### E. Preparation of Rat Pituitary Standard

1.) Prior to use, reconstitute the standard with 250  $\mu\text{L}$  deionized water. Invert the vial several times to mix. Allow the vial to set for 5-10 minutes and vortex. This will be used as Standard 6.

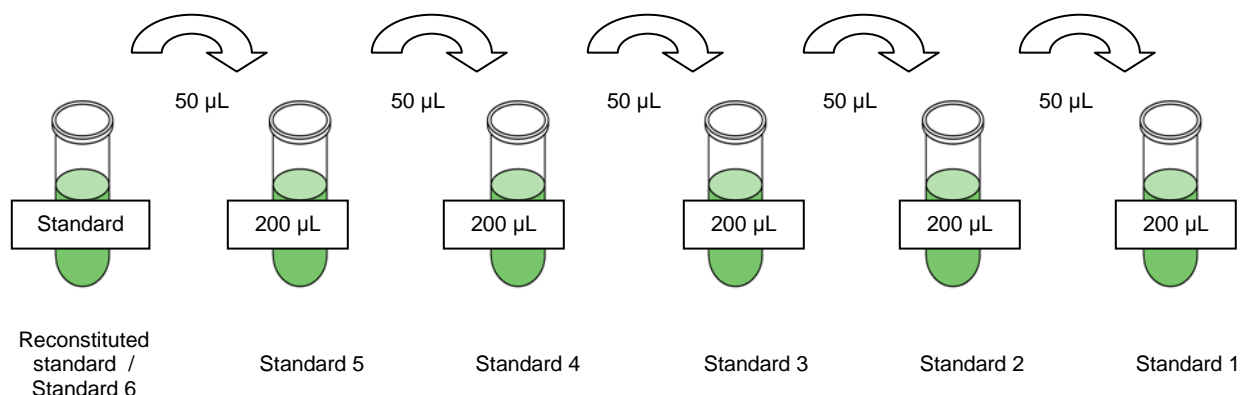
#### 2.) Preparation of Working Standards

Label five microfuge tubes Standard 1, Standard 2, Standard 3, Standard 4, and Standard 5. Add 200  $\mu\text{L}$  of Assay Buffer to each of the five tubes. Prepare serial dilutions by adding 50  $\mu\text{L}$  of the original reconstituted Standard 6 to the Standard 5 tube, mix well and transfer 50  $\mu\text{L}$  of the Standard 5 to the Standard 4 tube, mix well and transfer 50  $\mu\text{L}$  of the Standard 4 to the Standard 3 tube, mix well and transfer 50  $\mu\text{L}$  of the Standard 3 to Standard 2 tube, mix well and transfer 50  $\mu\text{L}$  of the standard 2 to the Standard 1 tube, mix well. The Standard 0 (Background) will be Assay Buffer.

### Preparation of Standards

Standard Concentration	Volume of Deionized Water to Add	Volume of Standard to Add
Standard 6 (Original)	250 $\mu\text{L}$	0

Standard	Volume of Assay Buffer to Add	Volume of Standard to Add
Standard 5	200 <input type="checkbox"/> $\mu\text{L}$	50 $\mu\text{L}$ of Standard 6
Standard 4	200 <input type="checkbox"/> $\mu\text{L}$	50 $\mu\text{L}$ of Standard 5
Standard 3	200 <input type="checkbox"/> $\mu\text{L}$	50 $\mu\text{L}$ of Standard 4
Standard 2	200 <input type="checkbox"/> $\mu\text{L}$	50 $\mu\text{L}$ of Standard 3
Standard 1	200 <input type="checkbox"/> $\mu\text{L}$	50 $\mu\text{L}$ of Standard 2



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

Standard Tube #	BDNF, ACTH, *LH, *TSH (pg/ml)	Prolactin, GH (pg/ml)	FSH (pg/ml)
1	3.2	16	32
2	16	80	160
3	80	400	800
4	400	2,000	4,000
5	2,000	10,000	20,000
6	10,000	50,000	100,000

**Note: Rat TSH and LH are calibrated against the reference preparations obtained from the National Hormone and Peptide Program (NHPP).**

## 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), 1, 2, 3, 4, 5, 6], 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.
- If using a filter plate, set the plate on a plate holder at all times during the reagent dispensing and incubation steps so the bottom of the plate does not touch any surface.

1. Add 200  $\mu$ L of Assay Buffer into each well of the plate. Seal and shake on a plate shaker for 10 minutes at room temperature (20-25°C)
2. Decant Assay Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
3. Add 25  $\mu$ L of each Standard or Control into the appropriate wells.
4. Add 25  $\mu$ L of Assay Buffer to the background and sample wells.
5. Add 25  $\mu$ L of appropriate matrix solution to the background, standards, and control wells. When assaying serum or plasma, use the 1:3 diluted Serum Matrix provided in the kit. When assaying tissue culture or other supernatant, use similar but hormone-free medium as the matrix solution.
6. Add 25  $\mu$ L of Sample into the appropriate wells. (Serum and plasma samples must be diluted 1:3 in diluted Serum Matrix.)
7. Vortex Mixing Bottle and add 25  $\mu$ L 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. Wrap the plate with foil and incubate with agitation on a plate shaker overnight (16-18 hours) at 4°C.

Add 200  $\mu$ L Assay Buffer per well



Shake 10 min, RT

Decant

- Add 25  $\mu$ L Standard or Control to appropriate wells
- Add 25  $\mu$ L Assay Buffer to background and sample wells
- Add 25  $\mu$ L appropriate matrix to background, standards and control wells
- Add 25  $\mu$ L Samples to sample wells
- Add 25  $\mu$ L Beads to each well



Incubate overnight (16-18 hours) at 4°C with shaking

9. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
10. Add 50  $\mu$ L of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)
11. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C). **DO NOT ASPIRATE AFTER INCUBATION.**
12. Add 50  $\mu$ L Streptavidin-Phycoerythrin to each well containing the 50  $\mu$ L of Detection Antibodies.
13. Seal, cover with foil and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
14. Gently remove well contents and wash plate 3 times following instructions listed in the **PLATE WASHING** section.
15. Add 100  $\mu$ L of Sheath Fluid (or Drive Fluid if using MAGPIX<sup>®</sup>) to all wells. Resuspend the beads on a plate shaker for 5 minutes.
16. Run plate on Luminex 200™, HTS or FLEXMAP 3D™, or MAGPIX<sup>®</sup> with xPONENT software.
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. (Note: For diluted samples, multiply the calculated concentration by the dilution factor.)



Remove well contents and wash 3X with 200  $\mu$ L Wash Buffer

Add 50  $\mu$ L Detection Antibodies per well



Incubate 30 minutes at RT  
Do Not Aspirate

Add 50  $\mu$ L Streptavidin-Phycoerythrin per well



Incubate for 30 minutes at RT



Remove well contents and wash 3X with 200  $\mu$ L Wash Buffer

Add 100  $\mu$ L Sheath Fluid (or Drive Fluid) per well

Read on Luminex (50  $\mu$ L, 50 beads per bead set)

## 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  $\mu$ L 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  $\mu$ L/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 (EMD Millipore Cat #MX-PLATE)

If using a filter plate, use a vacuum filtration manifold to remove well contents. Wash plate with 200  $\mu$ L/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

## EQUIPMENT SETTINGS (continued)

### Dispense:

1. Dispense volume: 200  $\mu$ L/well
2. Dispense flow rate: 5
3. Dispense height: 130 (16.51 mm)
4. Horizontal disp pos: 00 (0 mm)
5. Disable Aspirate: YES
6. Bottom Wash first?: NO
7. 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  $\mu$ l 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.**

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

These specifications are for the Luminex 200™, Luminex HTS and Luminex FLEXMAP 3D™ 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 (EMD Millipore Cat.# 40-275) and performance verified with the Performance Verification Kit (EMD Millipore Cat. # 40-276). The Luminex FLEXMap 3D™ instrument must be calibrated with the FLEXMAP 3D™ Calibrator Kit (EMD Millipore cat#40-028) and performance verified with the FLEXMAP 3D™ Performance Verification Kit (EMD Millipore cat#40-029). The Luminex MAGPIX® instrument must be calibrated with the MAGPIX® Calibration Kit (EMD Millipore cat# 40-049) and performance verified with the MAGPIX® Performance Verification Kit (EMD Millipore cat# 40-050).

**NOTE: These assays cannot be run 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:	7-Plex Beads	
	13	RACTH-MAG
	15	RBDNF-MAG
	26	RFSH-MAG
	28	RGH-MAG
	43	RLH-MAG
	45	RPRL-MAG
	62	RTSH-MAG



## 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 EMD MILLIPORE website [www.millipore.com/techlibrary/index.do](http://www.millipore.com/techlibrary/index.do) using the catalog number as the keyword.

## ASSAY CHARACTERISTICS

### Assay Sensitivities (minimum detectable concentrations)

MinDC: Minimum Detectable Concentration is calculated by the Milliplex Analyst Immunoassay Analysis Software. 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.

Pituitary Hormone (pg/mL)	Minimum DC Average + 2SD
ACTH	1.95
BDNF	0.38
FSH	7.62
GH	6.50
LH	3.28
Prolactin	4.64
TSH	0.87

### Precision

Intra-assay precision is generated from the mean of the %CV's from eight reportable results across two different concentrations of pituitary hormones in one experiment. Inter-assay precision is generated from the mean of the %CV's from one reportable result each for two different concentrations of pituitary hormones across six different experiments.

Pituitary Hormone	Intra-assay Precision (%CV)	Inter-assay Precision (%CV)
ACTH	2.5	12.1
BDNF	1.7	12.2
FSH	2.8	12.3
GH	3.1	18.3
LH	3.3	12.8
Prolactin	3.3	11.5
TSH	3.7	9.5

## Accuracy

Spike Recovery: The data represent mean percent recovery of three levels of pituitary hormones spiked into serum matrix

Pituitary Hormone	% Recovery
ACTH	88
BDNF	102
FSH	92
GH	75
LH	89
Prolactin	85
TSH	90

## Cross-Reactivity

Antibody pairs in the panel are specific and demonstrate no cross-reactivity (<0.1%) with other analytes in the panel except for LH which has ~5% cross-reactivity with antibody pair for TSH.

## 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.
	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°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	Keep plate and bead mix covered with dark

	light	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 removed, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been removed 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 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.
<b>FOR FILTER PLATES ONLY</b>		
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

Rat Pituitary Standard  
Rat Pituitary Quality Controls 1 & 2  
Rat Pituitary Detection Antibodies  
Streptavidin-Phycoerythrin  
Serum Matrix  
Bead Diluent  
Assay Buffer  
10X Wash Buffer  
Set of two 96-Well Plates with Sealers

## Catalog #

LRPT-8086  
LRPT-6086  
LRPT-1086  
L-SAPE  
LRPT-SM  
LHE-BD  
LE-ABGLP  
L-WB  
MAG-PLATE

## Antibody-Immobilized Beads

<u>Analyte</u>	<u>Bead #</u>	<u>Cat. #</u>
ACTH	13	RACTH-MAG
BDNF	15	RBDNF-MAG
FSH	26	RFSH-MAG
GH	28	RGH-MAG
LH	43	RLH-MAG
PRL	45	RPRL-MAG
TSH	62	RTSH-MAG

## ORDERING INFORMATION

### To place an order:

To assure the clarity of your custom rat pituitary hormone 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<sup>®</sup> MAG Rat Pituitary Panel Analytes/Serum Matrix Requirements

FAX: (636) 441-8050

Toll Free US: (800) MILLIPORE

MAIL ORDERS: EMD 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](mailto:customerserviceEU@Millipore.com).

### Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

### Material Safety Data Sheets (MSDS)

Material Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at [www.millipore.com/techlibrary/index.do](http://www.millipore.com/techlibrary/index.do)

### Technical Services

For product technical assistance call or write.

Toll-Free US: (781) 533-8159

E-mail: [techserv.dd@merckgroup.com](mailto:techserv.dd@merckgroup.com)

## WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 Standard (Background)	Standard 4	QC-2 Control									
B	0 Standard (Background)	Standard 4	QC-2 Control									
C	Standard 1	Standard 5	Sample 1									
D	Standard 1	Standard 5	Sample 1									
E	Standard 2	Standard 6	Sample 2									
F	Standard 2	Standard 6	Sample 2									
G	Standard 3	QC-1 Control	Etc.									
H	Standard 3	QC-1 Control										