Human Neurodegenerative Disease Panel 4

96-Well Plate Assay

Cat. #HNDG4-36K

MILLIPLEX® MAP

HUMAN NEURODEGENERATIVE DISEASE PANEL 4 KIT
96 Well Plate Assay

#HNDG4-36K

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

INTRODUCTION

Neurodegenerative disease is a condition characterized by the deterioration of neurons or their myelin sheath over time in the brain and/or spinal cord. These neurons are responsible for such everyday activities as processing sensory information, making decisions, and controlling movement. Because these cells are not easily regenerated, excessive cumulative damage can lead to age-related diseases such as Alzheimer's and Parkinson's disease, as well as other conditions such as amyotrophic lateral sclerosis (ALS) and epilepsy. These disorders are devastating and expensive, both on a personal and global level, and as population demographics continue to change, a therapeutic solution is critical. Consequently, research is underway to identify biomarkers that will help scientists not only understand the pathogenesis of neurodegenerative disease, but also identify people with these disorders before the onset of symptoms and potentially provide new therapeutic tools.

Therefore, understanding neurobiology is fundamental to determining the pathogenesis of these devastating neurodegenerative diseases. Identification of key biomarkers and their accurate measurement is crucial. However, conventional methods, including RIAs and ELISAs, are not able to simultaneously measure multiple biomarkers with small sample volume. The Luminex®-based Millipore's MILLIPLEX® MAP Human Neurodegenerative Panels will allow you to explore complexities of the nervous system and the pathobiology of disease.

Millipore's MILLIPLEX® MAP Human Neurodegenerative Disease Panel 4 is a versatile system available for neurobiology research.

- MILLIPLEX® MAP offers you the ability to:
    - Choose any combination of analytes from our panel of 5 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.

Millipore's MILLIPLEX® MAP Human Neurodegenerative Panel 4 kit is to be used for the simultaneous quantification of the following 5 analytes in any combination in human cerebrospinal fluid: S100B, Amyloid β40 (Aβ40), Amyloid β42 (Aβ42), RAGE, and GDNF. This kit is not suitable for testing serum or cell culture supernatants.

This kit is for research purposes only.

Please read entire protocol before use.

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

PRINCIPLE

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

- Luminex uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, 100 distinctly colored bead sets can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The microspheres are allowed to pass rapidly through a laser which excites the internal dyes marking the microsphere set. A second laser excites PE, the fluorescent dye on the reporter molecule.
- Finally, high-speed digital-signal processors identify each individual microsphere and quantify the result of its bioassay based on fluorescent reporter signals.

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

STORAGE CONDITIONS UPON RECEIPT

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

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8°C

REAGENTS SUPPLIED	CATALOG NUMBER	VOLUME	QUANTITY
Human Neurodegenerative Disease Panel 4 Standard	HNDG4-8036-4	lyophilized	1 vial
Human Neurodegenerative Disease Panel 4 Quality Controls 1 and 2	HNDG4-6036-4	lyophilized	2 vials
Bead Diluent	LBD-5	3.5 mL	1 bottle
Human Neurodegenerative Disease Panel 4 Aβ 40/42 Detection Antibody	HNDG4-ABETA	3.2 mL	1 bottle (required for Aβ40/42 only)
Human Neurodegenerative Disease Panel 4 Detection Antibodies	HNDG4-1036-4	**3.2 mL**	1 bottle
Set of two 96-Well Microtiter Filter Plates with 4 Sealers	MX-PLATE	----------	2 plates 4 sealers
Assay Buffer	L-AB3	30 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE9	3.2 mL	1 bottle
Mixing Bottle	----------	----------	1 bottle

Human Neurodegenerative Disease Panel 4 Antibody-Immobilized Beads:

Bead/Analyte Name	Luminex Bead Region	Customizable Beads (20X Concentration, 200 µL)	
		Available	Cat. #
Anti - S100B Bead	21	✓	HS100B
Anti - Aβ40 Bead	49	✓	HAB40
Anti - Aβ42 Bead	57	✓	HAB42
Anti - GDNF Bead	66	✓	HGDNF
Anti -s RAGE Bead	81	✓	HN4RAGE

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

SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, sodium azide and proclin may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

TECHNICAL GUIDELINES

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with an opaque plate lid or aluminum foil during all incubation steps.

TECHNICAL GUIDELINES (Continued)

- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- The bottom of the Microtiter Filter Plate should not come into direct contact with any surface during assay set-up or incubation times. The plate can be set on a plate stand or on the non-flat side of the plate cover, or any other plate holder to raise the plate from any surface. A plate stand can be purchased separately from Millipore. (Millipore Corporation, Catalog #MX-STAND).
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- After the wash steps, keep the bottom of the Microtiter Filter Plate clean by blotting on paper towels or absorbent pads to prevent any leakage due to capillary action.
- Keep the vacuum suction on the plate as low as possible. It is recommended to have a vacuum setting that will remove 200 µL of buffer in $\geq$ 5 seconds (equivalent to <100 mmHg).
- After hydration, all Standards and Controls must be transferred to polypropylene tubes.
- The Standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at ≤ -20°C for 1 month and at ≤ -80°C for greater than one month.
- If sample values 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 Mixing Bottle at 2-8°C for up to one month.
- During the preparation of the working standards, be certain to mix the higher concentration well before making the next dilution. In addition, use a new tip for 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.
- The titer plate shaker should be set at a speed that provides 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.
- Prior to reading the plate, ensure that the needle probe of the Luminex® apparatus is not clogged. This may be achieved by sonication and/or alcohol flushes. In addition, adjust the probe height according to the protocols recommended by Luminex Corporation. Briefly, adjust to the kit filter plate using 3 alignment discs prior to reading the plate.
- Vortex all reagents well before adding to plate.

SAMPLE STORAGE AND PREPARATION

This kit measures S100B, Aβ40, Aβ42, sRAGE, and GDNF in cerebrospinal fluid samples.

- Because Amyloid ß peptides tend to aggregate, preanalytic sample preparation is a critical influencing parameter within the analysis of Amyloid peptides. Samples should be collected according to clinical approved standard procedures and immediately stored at or below -20°C.
- Store the samples in small aliquots to avoid repeated freeze/thaw cycles. Repeated freeze/thaw cycles will result in inaccurate measurement of sample concentration.
- Insoluble material should be removed from all samples by centrifugation (4000xg for 10 minutes at 4°C) before testing.
- Samples should be kept on ice for the duration of the assay set up procedure.
- Customers need to determine the optimal dilution factor for their samples. Generally, CSF samples from normal subjects should be diluted 1:3 using the Assay Buffer provided in the kit as the sample diluent (e.g. Add 20 µL sample to 40 µL Assay Buffer). This dilution should occur immediately before loading the sample on the plate.

NOTE:

- A maximum of 25 µL per well of 1:3 diluted CSF sample can be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.

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 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 portion may be stored at 2-8°C for up to one month.

Example 1: 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.

Example 2: When using 5 antibody-immobilized beads, add 150 µL from each of the 5 bead sets to the Mixing Bottle. Then add 2.25 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 gently vortex. Allow the vial to sit for 5-10 minutes and then transfer the controls to appropriately labeled polypropylene microfuge tubes. Unused portion may be stored at ≤ -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 Human Neurodegenerative Disease Panel 4 Standard

1.) Prior to use, reconstitute the Human ND Panel 4 Standard with 250 µL deionized water (refer to table below for analyte concentration). Invert the vial several times to mix. Allow the vial to sit for 5 minutes for the standards to be completely reconstituted then invert the vial several times and vortex gently. Transfer the standard to an appropriately labeled polypropylene microfuge tube. This will be used as Standard 7. The unused portions may be stored at ≤ -20°C for up to one month.

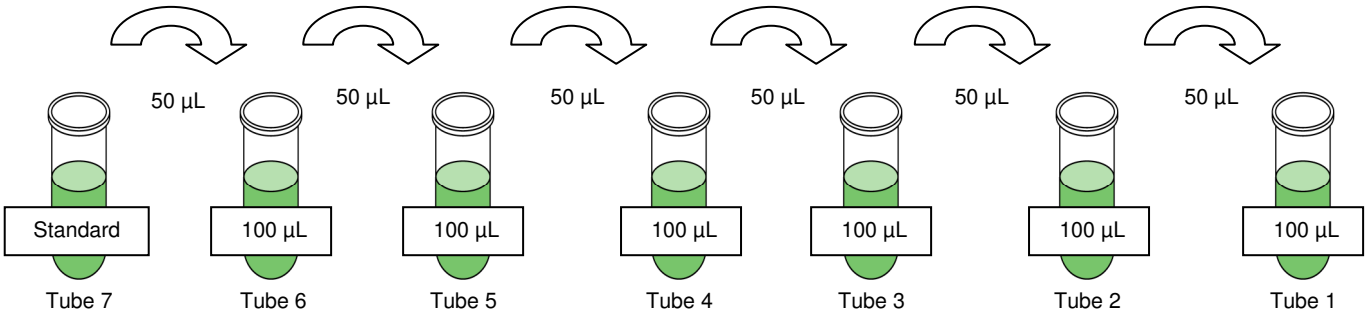
2.) Preparation of Working Standards

Label six polypropylene microfuge tubes as Standard 6, Standard 5, Standard 4, Standard 3, Standard 2, and Standard 1. Add 100 µL of Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 50 µL of the reconstituted Standard 7 to the Standard 6 tube, mix well and transfer 50 µL of the Standard 6 to the Standard 5 tube, mix well and transfer 50 µL of the Standard 5 to the Standard 4 tube, mix well and transfer 50 µL of the Standard 4 to the Standard 3 tube, mix well and transfer 50 µL of the Standard 3 to the Standard 2 tube, mix well and transfer 50 µL of the Standard 2 to the Standard 1 tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Standard (Tube #)	Volume of Deionized Water to Add	Volume of Standard To Add
Standard 7 (reconstituted standard)	250 µL	0

Standard (Tube #)	Volume of Assay Buffer to Add	Volume of Standard to Add
Standard 6	100 µL	50 µL of Standard 7
Standard 5	100 µL	50 µL of Standard 6
Standard 4	100 µL	50 µL of Standard 5
Standard 3	100 µL	50 µL of Standard 4
Standard 2	100 µL	50 µL of Standard 3
Standard 1	100 µL	50 µL of Standard 2

Preparation of Standards



After dilution, each tube has the following concentrations for each analyte:

Standard	S100B (pg/ml)	Aβ40 (pg/ml)	Aβ42 (pg/ml)	GDNF (pg/ml)	sRAGE (pg/ml)
Background	0	0	0	0	0
Standard 1	13.7	3.4	27.4	6.9	20.6
Standard 2	41.2	10.3	82.3	20.6	61.7
Standard 3	123.5	30.9	246.9	61.7	185.2
Standard 4	370.4	92.6	740.7	185.2	555.6
Standard 5	1111.1	277.8	2222.2	555.6	1666.7
Standard 6	3333.3	833.3	6666.7	1666.7	5000
Standard 7	10000	2500	20000	5000	15000

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 *except the samples* to warm to room temperature (20-25°C) before use in the assay.
- Keep the samples on ice during the entire assay procedure to achieve high stability and optimal data results. Dilute the samples 1:3 just before adding to the plate.
- All standards or samples should be mixed gently just before pipetting. Accurate mixing and pipetting of the standard solutions is essential to the precision of the assay.
- Diagram the placement of Background, Standards 1 through 7, Controls 1 and 2, and Samples on the 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 the bottom of the plate does not touch any surface.

1. Prewet the filter plate by pipetting 200 µL of Assay Buffer into each well of the Microtiter Filter Plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25℃).

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 Assay Buffer to Background wells.

4. Add 25 µL of each Standard or Control into the appropriate wells.

5. Add 25 µL of 1:3 diluted sample into the appropriate wells.

6. Add 25 µL of Aβ Detection Antibody (Cat# HNDG4-ABETA) to each well only when Aβ40 and/or Aβ42 beads are included in the analysis. If Aβ40 and Aβ42 beads are ***not*** included in the analysis, add 25 µL of Assay Buffer to each well.

7. Vortex Mixing Bottle and add 25 µL of the Mixed Beads to each well. (Note: During addition of Mixed Beads, shake Mixing Bottle intermittently to avoid settling.)

8. Seal the plate with a plate sealer then cover it with the opaque lid. Wrap a rubber band around the plate holder, plate and lid and incubate with agitation on a plate shaker overnight (16-20 hours) at 4℃.

9. Gently remove fluid by vacuum. (**NOTE: DO NOT INVERT PLATE).**

Add 200 µL Assay Buffer per well

Shake 10 min, RT
Vacuum

- Add 25 µL Assay Buffer to Background wells
- Add 25 µL Standard or Control to appropriate wells
- Add 25 µL Sample to sample wells
- Add 25 µL Aβ Detection Antibody (Cat# HNDG4-ABETA) only when Aβ40 and/or 42 beads are included or Assay Buffer to each well
- Add 25 µL Beads to each well

Incubate overnight
at 4℃ with shaking

10. Wash plate 3 times with 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Blot excess Wash Buffer from the bottom of the plate with an absorbent pad or paper towels.
11. Add 25 µL of Detection Antibodies (Cat# HNDG4-1036-4) into each well only when S100B, GDNF, and sRAGE beads are included in the analysis. If S100B, GDNF, and sRAGE beads are ***not*** included in the analysis, add 25 µL of Assay Buffer to each well **(Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)**.
12. Seal, cover with the opaque lid, and incubate with agitation on a plate shaker for 1.5 hours at room temperature (20-25°C) If S100B, GDNF, and sRAGE beads are ***not*** included, no incubation is necessary. **DO NOT WASH AFTER INCUBATION.**
13. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies or Assay Buffer.
14. Seal, cover with the opaque lid, and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
15. Gently remove fluid by vacuum. **(NOTE: DO NOT INVERT PLATE.)**
16. Wash plate 3 times with 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum between each wash.
17. Add 100 µL of Sheath Fluid to all wells. Resuspend the beads by incubating with agitation on a plate shaker for 5 minutes at room temperature.
18. Run plate on Luminex 100™IS, 200™, HTS.
19. Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method to calculate analyte concentrations in diluted samples. Multiply calculated concentrations by the dilution factor to determine sample concentration.

Vacuum and wash 3X with 200 µL Wash Buffer

Add 25 µL Detection Antibodies (Cat# HNDG4-1036-4) only when S100B, GDNF, and sRAGE beads are included or Assay Buffer to each well

Incubate 1.5 hours at RT only when S100B, GDNF, and sRAGE are included.

No incubation is necessary if S100B, GDNF, and sRAGE are ***not*** included.

Do Not Aspirate/vacuum

Add 25 µL Streptavidin-Phycoerythrin per well

Incubate for 30 minutes at RT

Aspirate/Vacuum and wash 3X with 200 µL Wash Buffer

Add 100 µL Sheath Fluid per well

Read on Luminex (50 µL, 50 beads per bead set)

EQUIPMENT SETTINGS

These specifications are for the Luminex 100™ IS v.1.7, Luminex 100™ IS v2.1/2.2, Luminex 200™ v2.3, xPONENT®, and Luminex HTS. Luminex instruments with other software (e.g. MasterPlex®, StarStation®, LiquiChip®, Bio-Plex®, LABScan®100) would need to follow instrument instructions for gate settings and additional specifications from the vendors.

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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:	5-Plex Beads	
	S100B	21
	Aβ40	49
	Aβ42	57
	GDNF	66
	sRAGE	81

QUALITY CONTROLS

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at the MILLIPORE website www.millipore.com/techlibrary/index.do using the catalog number as the keyword.

ASSAY CHARACTERISTICS

Cross-Reactivity

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

Assay Sensitivities (minimum detectable concentrations, pg/mL)

MinDC: Minimum Detectable Concentration is calculated using the Milliplex Analyst Software from Millipore. 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 in an overnight assay.

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Analyte	***MinDC + 2SD (pg/ml)***
S100B	7.3
Aβ40	2.0
Aβ42	7.5
GDNF	1.9
sRAGE	6.8

N=8 assays

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentrations of analytes in a single assays. Inter-assay precision is generated from the mean of the %CV's across two different concentrations of analytes across six different assays.

Analyte	***Intra-Assay (%CV)***	***Inter-Assay (%CV)***
S100B	7.3	8.3
Aβ40	5.5	7.5
Aβ42	13.0	17.1
GDNF	6.0	6.3
sRAGE	6.4	9.1

Accuracy

Spike Recovery: The data represent mean percent recovery of 3 levels of spiked standards in cerebrospinal fluid samples (n=5).

	Spike Recovery (%) in CSF
Analyte	***Overnight Protocol***
S100B	96
Aβ40	96
Aβ42	89
GDNF	101
sRAGE	97

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	With Luminex 200™, adjust probe height according to the protocols recommended by Luminex to the kit solid plate using 4 alignment discs. With FLEXMAP 3D™, adjust probe height according to the protocols recommended by Luminex to 0.74" for the kit solid plate.
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 pipetting with Multichannel pipettes without touching reagent in plate
	Matrix used has endogenous analyte or	Check matrix ingredients for cross reacting components (e.g. interleukin modified tissue

	interference Insufficient washes	culture medium) Increase number of washes
Beads not in region or gate	Luminex not calibrated correctly or recently Gate Settings not adjusted correctly Wrong bead regions in protocol template Incorrect sample type used Instrument not washed or primed Beads were exposed to light	Calibrate Luminex based on Instrument Manufacturer's instructions, at least once a week or if temperature has changed by >3^{o}C Some Luminex instruments (e.g. Bioplex) require different gate settings than those described in the Kit protocol. Use Instrument default settings. Check kit protocol for correct bead regions or analyte selection Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required 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. 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 Streptavidin-Phycoerythrin was not added	Add appropriate Detection Antibody and continue 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 Incubations done at inappropriate temperatures, timings or agitation	May need to repeat assay if desired sensitivity not achieved Assay conditions need to be checked.
Signals too high, standard curves are saturated	Calibration target value set too high Plate incubation was too long with standard curve and samples	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 Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte Samples contain analyte concentrations higher than highest standard point. Standard curve was saturated at higher end of curve.	If below detectable levels, it may be possible to use higher sample volume. Check with tech support for appropriate protocol modifications. Samples may require dilution and reanalysis for just that particular analyte See above

High Variation in samples and/or standards	Multichannel pipette may not be calibrated	Calibrate pipettes
	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 pipette tips that are used for reagent additions and that pipette tip does not touch reagent in plate.

REPLACEMENT REAGENTS

Components	**Cat #**
Human Neurodegenerative Disease Panel 4 Standard	HNDG4-8036-4
Human Neurodegenerative Disease Panel 4 Quality Controls	HNDG4-6036-4
Human Neurodegenerative Disease Panel 4 Aβ Detection Antibody	HNDG4-ABETA
Human Neurodegenerative Disease Panel 4 Detection Antibodies	HNDG4-1036-4
Streptavidin-Phycoerythrin	L-SAPE9
Assay Buffer	L-AB3
Bead Diluent	LBD-5
Set of two 96-Well Plates with Sealers	MX-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Magnetic Beads

Analyte	Bead #	Cat. #
S100B	21	HS100B
Aβ40	49	HAB40
Aβ42	57	HAB42
GDNF	66	HGDNF
sRAGE	81	HN4RAGE

ORDERING INFORMATION

To place an order:

To assure the clarity of your custom Human Neurodegenerative Disease Panel 4 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® *MAP* Human Neurodegenerative Disease Panel 4 Analyte Requirements

FAX: (636) 441-8050
Toll-Free US: (800) MILLIPORE
Mail Orders: Millipore Corp.
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers in placing an order or obtaining additional information about MILLIPLEX® *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 pg/mL Standard (Background)	Standard 4	QC-1 Control	Etc.								
B	0 pg/mL 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									