MILLIPLEX™ MAP

HUMAN RANKL SINGLE PLEX KIT
96 Well Plate Assay

HBN51K1RANKL

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

INTRODUCTION

Bone metabolism is the dynamic process of ongoing bone deposition and resorption, controlled by osteoblasts, osteocytes, and osteoclasts. While osteoblasts and osteocytes (osteoblasts surrounded by matrix) are responsible for bone deposition, osteoclasts are responsible for bone resorption. Both are required to maintain bone structure, as well as an adequate supply of calcium. To maintain this metabolic balance these cells rely on complex signaling pathways involving hormones and cytokines to achieve the appropriate rates of growth and differentiation. One of these proteins, Receptor Activator for Nuclear Factor κB Ligand (RANKL) activates osteoclasts and has been implicated in degenerative bone diseases such as rheumatoid arthritis and osteomyelitis. The balance between OPG and RANKL regulates osteoclast differentiation, activation and survival.

Millipore recognizes the need to better understand the role that bone metabolism biomarkers like RANKL play both in preserving normal bone structure and in the development of disease. Therefore, we are proud to announce that the former LINCO*plex* Human Bone Metabolism RANKL Single Plex now has the MILLIPLEX MAP optimized format. While you will immediately recognize the quality and reproducibility that you have always trusted, you will also enjoy the enhancements that we have built into MILLIPLEX MAP.

Millipore's MILLIPLEX MAP Human Bone Metabolism RANKL Single Plex is to be used for the quantification of RANKL in human tissue, plasma and cell / tissue culture supernatant samples. The single plex provides biomedical researchers with quality tools for the study of bone metabolism related diseases.

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 RANKL Standard	LHBN-8051-RANKL	lyophilized	1 vial
Human RANKL Quality Controls 1 and 2	LHBN-6051-RANKL	lyophilized	2 vials
Serum Matrix Note: Contains 0.08% Sodium Azide	LHED-SD	lyophilized	1 vial (required for serum and plasma samples only)
Bead Diluent	LPX-BD	3.5 mL	1 bottle
Set of one 96-Well Filter Plate with 2 Sealers	MX-PLATE	----------	1 plate 2 sealers
Assay Buffer	L-AB1	30 mL	2 bottles
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	1 bottle
Human RANKL Detection Antibody	LHBN1051RANKL	5.5 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE	5.5 mL	1 bottle
Mixing Bottle	----------	----------	1 bottle

Bead/Analyte Name	Luminex Bead Region	(20X concentration, 200µL) Available	Cat. #
Anti-Human RANKL Bead	14	✓	HRANKL

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

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

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 5 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™, or HTS by Luminex Corporation
12. Plate Stand (Millipore Catalog # MX-STAND)

SAFETY PRECAUTIONS

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

TECHNICAL GUIDELINES

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

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.

- The bottom of the Microtiter Filter Plate should not be in direct contact with any surface during assay set-up or incubation times. The plate can be set on a plate stand or on the non-flat side of the plate cover or any other plate holder to raise the plate from the surface. A plate stand can be purchased separately from Millipore (Millipore Catalog #MX-STAND).
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- After the wash steps, keep the bottom of the Microtiter Filter Plate clean by blotting on paper towels or absorbent pads to prevent any leakage due to capillary action.
- Keep the vacuum suction on the plate as low as possible. It is recommended to have a vacuum setting that will remove 200 µL of buffer in ≥ 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 samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- Any unused mixed Antibody-Immobilized Beads may be stored in the Mixing Bottle at 2-8°C for up to one month.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some analytes.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes. Adjust probe height according to the protocols recommended by Luminex to the kit filter plate using 3 alignment discs prior to reading an assay.
- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in background, standard curve and control wells. If samples are diluted in Assay Buffer, use the Assay Buffer as matrix.
- For serum/plasma samples, use the Serum Matrix provided in the kit.
- For cell/tissue homogenate, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Avoid debris, lipids, and cell/tissue aggregates. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°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.
- **Samples should not be diluted until immediately prior to setting up the assay.**
- Serum samples should be diluted 1:2 in the Assay Buffer provided in the kit.

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 ≤ -20°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.
- **Samples should not be diluted until immediately prior to setting up the assay.**
- Plasma samples should be diluted 1:2 in the Assay Buffer provided in the kit.

C. Preparation of Tissue / Cell Culture Supernatant:

- Centrifuge the sample for 10 minutes at 1000xg to remove debris and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- Customers may need to determine the optimal dilution factors for their tissue culture supernatant samples depending on the expected biological range. (If dilution is required, **samples should not be diluted until immediately prior to setting up the assay**.)

NOTE:

- A maximum of 25 µL per well of cell / tissue culture supernatant samples or neat serum, plasma samples can be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid 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

Pre-warm the Bead Diluent to room temperature. Sonicate the antibody-bead tube for 30 seconds, and then vigorously vortex the tube for 1 minute. Add 150 µL of RANKL beads to the Mixing Bottle and add 2.85 mL of Bead Diluent to obtain 3.0 mL of diluted beads. Mix the diluted beads well immediately prior to use.

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 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 Serum Matrix

This step is required for serum or plasma samples only.

Before the assay, add 1 mL of deionized water and 4 mL of L-AB1 Assay Buffer to the bottle containing the lyophilized Serum Matrix, gently swirl the bottle and then place the bottle on bench for 10 minutes. Transfer the reconstituted Serum Matrix solution to an appropriately-labeled polypropylene tube. Unused portion may be stored at ≤ -20°C for up to one month.

E. Preparation of Human RANKL Standard

1.) Prior to use, reconstitute the lyophilized Human RANKL Standard with 250 µL Deionized Water to give a concentration of 20,000 pg/mL for RANKL. Invert the vial several times to mix. Allow the vial to set for 5-10 minutes to make sure that the standard is completely reconstituted, and then transfer the standard to an appropriately-labeled polypropylene microfuge tube. This will be used as the 20,000 pg/mL Standard. The unused portions of this stock may be stored at ≤ -20°C for up to one month.

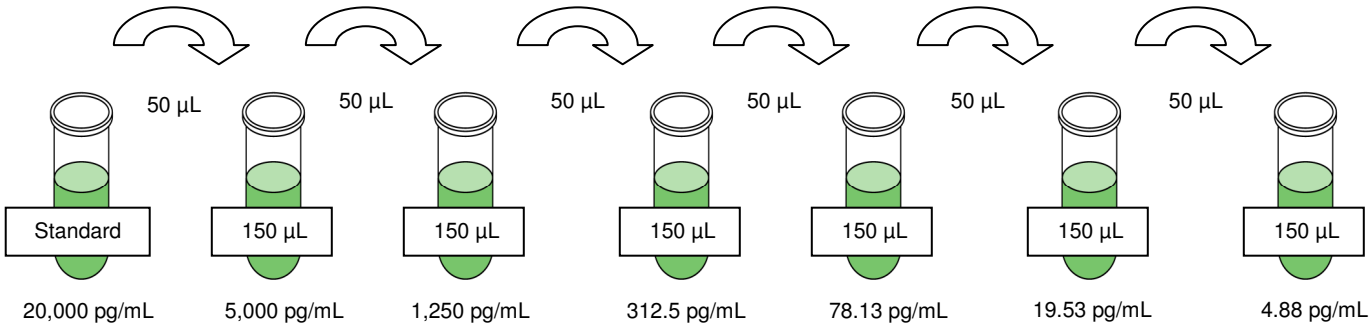
2.) Preparation of Working Standards

Label six polypropylene microfuge tubes as 5,000, 1,250, 312.5, 78.13, 19.53, and 4.88 pg/mL. Add 150 µL of Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 50 µL of the reconstituted 20,000 pg/mL standard to the 5,000 pg/mL tube, mix well and transfer 50 µL of the 5,000 pg/mL standard to the 1,250 pg/mL tube, mix well and transfer 50 µL of the 1,250 pg/mL standard to the 312.5 pg/mL tube, mix well and transfer 50 µL of the 312.5 pg/mL standard to the 78.13 pg/mL tube, mix well and transfer 50 µL of the 78.13 pg/mL standard to the 19.53 pg/mL tube, mix well and transfer 50 µL of the 19.53 pg/mL standard to the 4.88 pg/mL tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Preparation of Working Standards

Standard Concentration	Volume of Deionized Water to Add	Volume of Standard to Add
Original 20,000 pg/mL	250 µL	0

Standard Concentration (pg/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
5,000	150 µL	50 µL of 20,000 pg/mL
1,250	150 µL	50 µL of 5,000 pg/mL
312.5	150 µL	50 µL of 1,250 pg/mL
78.13	150 µL	50 µL of 312.5 pg/mL
19.53	150 µL	50 µL of 78.13 pg/mL
4.88	150 µL	50 µL of 19.53 pg/mL



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), 4.88, 19.53. 78.13, 312.5, 1250, 5,000 and 20,000 pg/mL], Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.
- Set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

1. Prewet the filter plate by pipetting 200 µL of Assay Buffer into each well of the Microtiter Filter Plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Remove Assay Buffer by vacuum. **(NOTE: DO NOT INVERT PLATE.)** Blot excess Assay Buffer from the bottom of the plate with an absorbent pad or paper towels.
3. Add 25 µL Assay Buffer to all wells.
4. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for the 0 pg/mL standard (Background).
5. Add 25 µL of Assay Buffer to the sample wells.
6. Add 25 µL of an appropriate matrix to the Background, Standards, and Control wells. When assaying 1:2 diluted Serum or Plasma samples, use the diluted Serum Matrix provided. When assaying Tissue Culture or other supernatant, use control culture medium as the matrix solution.
7. Add 25 µL of Sample into the appropriate wells. (Serum and plasma samples should be diluted 1:2 in Assay Buffer immediately prior to setting up assay.)
8. Vortex Mixing Bottle and add 25 µL of the Mixed Beads to each well. (Note: During addition of Beads, shake bead bottle intermittently to avoid settling.)
9. Seal the plate with a plate sealer, cover it with the lid. Wrap a rubber band around the plate holder, plate and lid and incubate with agitation on a plate shaker overnight (16-20 hours) at 4°C.

Add 200 µL Assay Buffer per well

↓ Shake 10 min, RT

↓ Vacuum

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

↓ Incubate overnight at 4°C with shaking

10. Gently remove fluid by vacuum. **(NOTE: DO NOT INVERT PLATE.)**
11. 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.
12. Add 50 µL of Detection Antibody into each well. (Note: Allow the Detection Antibody to warm to room temperature prior to addition.)
13. Seal, cover with lid, and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C). **DO NOT VACUUM AFTER INCUBATION.**
14. Add 50 µL Streptavidin-Phycoerythrin to each well containing the 50 µL of Detection Antibodies.
15. Seal, cover with lid and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
16. Gently remove all contents by vacuum. **(NOTE: DO NOT INVERT PLATE.)**
17. Wash plate 3 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Wipe any excess buffer on the bottom of the plate with a tissue.
18. Add 100 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.
19. Run plate on Luminex 100™ IS, 200™, or HTS.
20. Save and analyze the Median Fluorescent Intensity (MFI) data using a weighted 5-parameter logistic or spline curve-fitting method for calculating RANKL concentrations in samples.

Vacuum and wash 3X with 200 µL Wash Buffer

Add 50 µL Detection Antibody per well

Incubate 1 hour at RT

Do Not Vacuum

Add 50 µL Streptavidin-Phycoerythrin per well

Incubate for 30 minutes at RT

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 or Luminex 100™ IS v2.1/2.2, Luminex 200™ v2.3, xPONENT, and Luminex HTS. Luminex instruments with other software (e.g. MasterPlex, StarStation, LiquiChip, Bio-Plex, LABScan100) would need to follow instrument instructions for gate settings and additional specifications from the vendors.

Events:	50, per bead	
Sample Size:	50 µL	
Gate Settings:	8,000 to 15,000	
Reporter Gain:	Default (low PMT)	
Time Out:	60 seconds	
Bead Set:	RANKL	14

QUALITY CONTROLS

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

ASSAY CHARACTERISTICS

Assay Sensitivities (minimum detectable concentrations, pg/mL)

MinDC: Minimum Detectable Concentration is calculated by the StatLIA® Immunoassay Analysis Software from Brendan Technologies. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions.

Analyte	MinDC (pg/mL)
RANKL	4.8

Precision

Intra-assay precision is generated from the mean of the %CV's from sixteen reportable results across two different concentrations of analyte 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 analyte across eight different experiments.

Intra-Assay (%CV)	<5%
Inter-Assay (%CV):	<6%

Accuracy

Spike Recovery: The data represent mean percent recovery of 3 levels of spiked standards in four independent serum samples.

% Recovery	93.2%

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 set-up 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 incorrectly	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with alcohol flush, backflush and washes; or, if needed, probe should be removed and sonicated.
	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 (prewetted) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate stand 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 well.
	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 by pipeting with multichannel pipets without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for crossreacting 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. Bio-Plex) 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 eliminate 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 incorrect 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 instruments (e.g. Bio-Plex) default target setting for RP1 calibrator is set at High PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with 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 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 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 RANKL Standard	LHBN-8051-RANKL
Human RANKL Quality Controls	LHBN-6051-RANKL
Human RANKL Detection Antibody	LHBN1051RANKL
Streptavidin-Phycoerythrin	L-SAPE
Bead Diluent	LPX-BD
Assay Buffer	L-AB1
Serum Matrix	LHED-SD
10X Wash Buffer	L-WB
Set of two 96-Well Filter Plates with Sealers	MX-PLATE

Antibody-Immobilized Bead

Analyte	Bead #	Cat. #
Human RANKL Beads	14	HRANKL

ORDERING INFORMATION

To place an order:

To assure the clarity of your custom Human RANKL kit order, please FAX the following information to our customer service department:

- Your name, telephone and/or fax number
- Customer account number
- Shipping and billing address
- Purchase order number
- Catalog number and description of product
- Quantity of kits

FAX: (636) 441-8050
Toll-Free US: (866) 441-8400
(636) 441-8400

Mail Orders: Millipore Corp.
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers in placing an order or obtaining additional information about MILLIPLEX™ MAP products, please contact your multiplex specialist or sales representative or email our European Customer Service at customerserviceEU@Millipore.com.

Conditions of Sale

All products are for research use only. They are not intended for use in clinical diagnosis or for administration to humans or animals. All products are intended for *in vitro* use only.

Material Safety Data Sheets (MSDS)

Material Safety Data Sheets for Millipore products may be ordered by fax or phone or through our website at www.millipore.com/techlibrary/index.do.

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WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 Standard (Background)	312.5 pg/mL Standard	QC 1	Etc.								
B	0 Standard (Background)	312.5 pg/mL Standard	QC 1									
C	4.88 pg/mL Standard	1,250 pg/mL Standard	QC 2									
D	4.88 pg/mL Standard	1,250 pg/mL Standard	QC 2									
E	19.53 pg/mL Standard	5,000 pg/mL Standard	Sample 1									
F	19.53 pg/mL Standard	5,000 pg/mL Standard	Sample 1									
G	78.13 pg/mL Standard	20,000 pg/mL Standard	Sample 2									
H	78.13 pg/mL Standard	20,000 pg/mL Standard	Sample 2									