Luminex® Screening Assay

Human Premixed Multi-Analyte Kit

Catalog Number VMAPH

For the simultaneous detection of multiple human biomarkers in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

This kit contains the components required to screen multiple human biomarkers in cell culture supernate, serum, and plasma samples in multiplexed sandwich ELISAs.

Luminex® Screening Assays can be used to assess the levels of biomarkers of your choosing in a single sample. For ease of use, the microparticles are premixed in one vial as are the biotinylated detection antibodies.

PRINCIPLE OF THE ASSAY

The Luminex Screening Assay is designed for use with Luminex 100™, Luminex 200™ or Bio-Rad® Bio-Plex®, dual laser, flow-based sorting and detection platforms.

Analyte-specific antibodies are pre-coated onto color-coded microparticles. Microparticles, standards, and samples are pipetted into wells and the immobilized antibodies bind the biomarkers of interest. After washing away any unbound substances, a biotinylated antibody cocktail specific to the biomarkers of interest is added to each well. Following a wash to remove any unbound biotinylated antibody, streptavidin-phycoerythrin conjugate (Streptavidin-PE), which binds the biotinylated detection antibodies, is added to each well. A final wash removes unbound Streptavidin-PE, and the microparticles are resuspended in buffer and read using the Luminex or Bio-Plex analyzer. One laser is microparticle-specific and determines which biomarker is being detected. The other laser determines the magnitude of the phycoerythrin-derived signal, which is in direct proportion to the amount of biomarker bound.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- Discepancies may exist in values obtained for the same analyte utilizing different technologies.
- Luminex Screening Assays afford the user the benefit of multianalyte analysis of biomarkers in a single sample. A multipurpose diluent is used to dilute samples, if necessary, and provide accurate estimates of natural analytes in cell culture supernates, serum, and plasma.
- **Only the analytes listed on the enclosed Certificate of Analysis can be measured with this kit.**

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Protect microparticles and Streptavidin-PE from light at all times to prevent photobleaching.
- For best results, adjust the vacuum strength on the plate washer to between 15 and 40 cm of mercury.

PRECAUTIONS

Some components in this kit contain ProClin® which may cause an allergic skin reaction. Avoid breathing mist.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

This kit contains sufficient materials to run multiplex assays on two 96 well plates.

PART	PART #	DESCRIPTION	STORAGE OF OPENED, DILUTED, OR RECONSTITUTED MATERIAL
Standard Cocktail A†	893899	2 vials of recombinant human biomarkers in a buffered protein base with preservatives; lyophilized.	Once reconstituted, any remaining standard must be discarded. Use fresh standard(s) for each assay.
Standard Cocktail B†	893901	2 vials of recombinant human biomarkers in a buffered protein base with preservatives; lyophilized.	
Standard Cocktail C†	893984	2 vials of recombinant human biomarkers in a buffered protein base with preservatives; lyophilized.	
Standard Cocktail D†	893985	2 vials of recombinant human biomarkers in a buffered protein base with preservatives; lyophilized.	
Standard Cocktail E†	893986	2 vials of recombinant human biomarkers in a buffered protein base with preservatives; lyophilized.	
Premixed Microparticle Cocktail	893987	1.2 mL of a concentrated human microparticle cocktail with preservatives.	May be stored for up to 1 month at 2-8 °C.* *Once diluted, these solutions must be discarded. Use fresh dilutions for each assay.*
Premixed Biotin Antibody Cocktail	893988	1.2 mL of a concentrated human biotin antibody cocktail with preservatives.	
Streptavidin-PE	892525	2 vials (0.07 mL/vial) of a 100X concentrated streptavidin-phycoerythrin conjugate with preservatives.	
Diluent RD2-1	895970	2 vials (11 mL/vial) of a buffered protein base with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Calibrator Diluent RD6-52	895438	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	May be stored for up to 1 month at room temperature.
Microplates	640763	2 filter-bottomed 96-well microplates used as vessels for the assay.	
Certificate of Analysis	752180	1 sheet listing the selected analytes with the microparticle regions, standard reconstitution volumes, and concentrations for the provided Standard(s).	
Mixing Bottles	895971	2 empty 15 mL bottles used for mixing microparticles with Diluent RD2-1.	
Plate Sealers	640445	6 adhesive foil strips.	

*Provided this is within the expiration date of the kit.

† Each premixed kit may contain 1 or more of the unique Standard Cocktails (A-E), depending upon the analytes selected.

OTHER SUPPLIES REQUIRED

- Luminex 100, Luminex 200, or Bio-Rad Bio-Plex analyzer with X-Y platform.
- Microplate vacuum manifold (Millipore Multiscreen™ Vacuum Manifold Catalog # MAVM096 or equivalent).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Multi-channel pipette, manifold dispenser, or automated dispensing unit.
- 50 mL and 500 mL graduated cylinders.
- **Polypropylene** test tubes for dilution of standards and samples.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Microcentrifuge.

SAMPLE COLLECTION & STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifuging for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *EDTA and citrate plasma have not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require at least a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD6-52. Mix thoroughly.

High abundance biomarkers may require additional dilution such as 50- or 200-fold.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

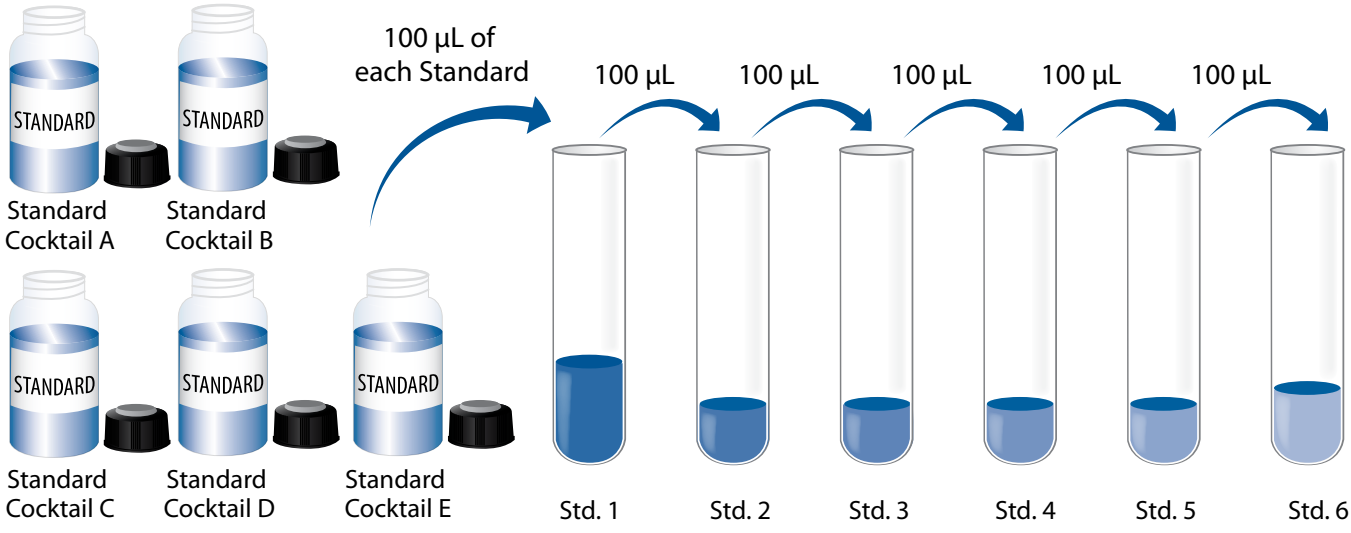
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer. If assaying a partial plate, prepare only as much Wash Buffer as needed.

Standards - The standards provided in the kit will differ depending on the analytes selected, but may include up to 5 unique Standard Cocktails (A-E). Reconstitute 1 each of the unique Standard Cocktails provided in the kit with Calibrator Diluent RD6-52. Refer to the Certificate of Analysis for reconstitution volumes. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Upon reconstitution, each Standard Cocktail is a 5X concentrate.

Use polypropylene tubes. Combine the Standard Cocktails with Calibrator Diluent RD6-52 according to the table below. This results in a single 1X Standard containing all of the selected analytes. Label this as Standard 1.

Number of Unique Standard Cocktails Provided	Volume to Combine into a Single Tube	Volume of Calibrator Diluent Required	Total Volume of Standard 1
1	100 µL	400 µL	500 µL
2	100 µL of each	300 µL	500 µL
3	100 µL of each	200 µL	500 µL
4	100 µL of each	100 µL	500 µL
5	100 µL of each	0 µL	500 µL

Pipette 200 µL of Calibrator Diluent RD6-52 into each of 5 test tubes labeled 2-6. Use Standard 1 to produce a 3-fold dilution series (below). Mix each tube thoroughly before the next transfer. Standard 1 serves as the high standard. Calibrator Diluent RD6-52 serves as the blank. Refer to the Certificate of Analysis for the assigned values of combined Standard 1.



DILUTED MICROPARTICLE COCKTAIL PREPARATION

1. Centrifuge the Microparticle Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial to resuspend the microparticles, taking precautions not to invert the vial.
3. Dilute the Microparticle Cocktail using Diluent RD2-1 in the mixing bottle provided.

Number of Wells Used	Microparticle Cocktail	+	Diluent RD2-1
192	1.00 mL	+	10.0 mL
144	750 µL	+	7.50 mL
96	500 µL	+	5.00 mL
48	250 µL	+	2.50 mL

Note: *Protect microparticles from light during handling. Prepare microparticles within 30 minutes of use.*

DILUTED BIOTIN ANTIBODY COCKTAIL PREPARATION

1. Centrifuge the Biotin Antibody Cocktail vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the Biotin Antibody Cocktail in Diluent RD2-1. Mix gently.

Number of Wells Used	Biotin Antibody Cocktail	+	Diluent RD2-1
192	1.00 mL	+	10.0 mL
144	750 µL	+	7.50 mL
96	500 µL	+	5.00 mL
48	250 µL	+	2.50 mL

STREPTAVIDIN-PE PREPARATION

Use a polypropylene amber bottle or a polypropylene test tube wrapped with aluminum foil. Protect the Streptavidin-PE from light during handling and storage.

1. Centrifuge the Streptavidin-PE vial for 30 seconds at 1000 x g prior to removing the cap.
2. Gently vortex the vial, taking precautions not to invert the vial.
3. Dilute the 100X Streptavidin-PE to a 1X concentration by adding 55 µL of Streptavidin-PE to 5.5 mL of Wash Buffer. This provides enough Streptavidin-PE to assay one 96-well microplate. If assaying more or less than 96 wells, adjust these volumes accordingly.

INSTRUMENT SETTINGS

Adjust the probe height setting on the Luminex analyzer to avoid puncturing the membrane. Refer to the instrument manual.

1. Assign the bead region for each analyte being measured (refer to the Certificate of Analysis)

2. 50 events/bead

3. Minimum events: 0

4. Flow rate: 60 µL/minute (fast)

5. Sample size: 50 µL

6. Doublet Discriminator gates at approximately 7500 and 15,500

7. Collect Median Fluorescence Intensity (MFI)

Note: *For the Bio-Rad Bio-Plex analyzer, set the gates at 4300 and 10,000. The CAL2 setting for the Bio-Rad Bio-Plex analyzer should be set at the low RP1 target value.*

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.

Note: *Protect microparticles and Streptavidin-PE from light at all times.*

1. Prepare all reagents, standards, and samples as directed in the previous sections.
2. Pre-wet the filter-bottomed microplate by filling each well with 100 µL of Wash Buffer. Remove the liquid through the filter at the bottom of the plate using a vacuum manifold designed to accomodate a microplate.

   Note: *After each final wash cycle and subsequent reagent addition, blot the bottom of the microplate with a paper towel to prevent wicking.*
3. Resuspend the diluted Microparticle Cocktail by inversion or vortexing. Add 50 µL of the mixture to each well of the pre-wet filter-bottomed microplate.
4. Add 50 µL of Standard or sample per well. Securely cover with a foil plate sealer. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Using a vacuum manifold device designed to accomodate a microplate, wash by removing the liquid, filling each well with Wash Buffer (100 µL), and removing the liquid again. All of the liquid must be removed through the filter at the bottom of the microplate to avoid any loss of microparticles. Complete removal of liquid is essential for good performance. Perform the wash procedure three times.
6. Add 50 µL of diluted Biotin Antibody Cocktail to all wells. Securely cover with a new foil plate sealer, and incubate for 1 hour at room temperature on the shaker set at 500 ± 50 rpm.
7. Repeat the wash as in step 5.
8. Add 50 µL of diluted Streptavidin-PE to all wells. Securely cover with a new foil plate sealer, and incubate for 30 minutes at room temperature on the shaker set at 500 ± 50 rpm.
9. Repeat the wash as in step 5.
10. Resuspend the microparticles by adding 100 µL of Wash Buffer to each well. Incubate for 2 minutes at room temperature on the shaker set at 500 ± 50 rpm.
11. Read within 90 minutes using the Luminex or Bio-Rad analyzer.

CALCULATION OF RESULTS

Use the Standard concentrations on the Standard Value Card and calculate 3-fold dilutions for the remaining levels. Average the duplicate readings for each standard and sample and subtract the average blank Median Fluorescence Intensity (MFI).

Create a standard curve for each analyte by reducing the data using computer software capable of generating a five parameter logistic (5-PL) curve-fit.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This assay is calibrated against highly purified recombinant human proteins produced at R&D Systems.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.