Milliplex Cardiovascular Disease 3-plex Panel 2

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Abstract

Luminex Milliplex Cardiovascular Disease 3-plex Panel 2 instructions per manufacturer.

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Protocol

Step 1.

PREPARATION OF SAMPLES/REAGENTS FOR IMMUNOASSAY

1. Preparation of Serum/Plasma **Thaw Time:**

Thaw the samples completely on ice, mix well by shaking on plate shaker for 1 min. at RT (20-25°C) and centrifuge (1,700 x g, 10 minutes, 4°C) prior to use in the assay to remove particulates. L-AB Assay Buffer provided in the kit should be used as the sample diluent.

Serum/Plasma sample dilution (1:2000): Step 1: add 20 µL serum/plasma to 980 µL Assay Buffer (1:50); Step 2: add 20 µL of 1:50 diluted sample to 780 µL of Assay Buffer (1:2,000).

1. 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: 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.

1. Preparation of Quality Controls **Reconstitution Time:**

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.*

1. 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.*

1. Preparation of Human CVD Panel 2 Standard **Reconstitution Time:**

1.) Prior to use, reconstitute the Human CVD Panel 2 Standard with 250 µL deionized

water to give a stock concentration termed STD7. Invert the vial several times to mix. Allow the vial to sit for 5-10 minutes and then transfer the standard to an appropriately labeled polypropylene microfuge tube. This will be used as the STD7 tube; the *unused portion may be stored at £ -20°C for up to one month*.

2.) Preparation of Working Standards. Label six polypropylene microfuge tubes STD6, STD5, STD4, STD3, STD2, and STD1.

Add 200 µL of Assay Buffer to each of the six tubes.

-Prepare serial dilutions by adding 50 µL of the STD7 reconstituted standard to the STD6 tube, mix well and transfer 50 µL of the STD6 standard to the STD5 tube, mix well and transfer 50 µL of the STD5 standard to the STD4 tube, mix well and transfer 50 µL of the STD4 standard to STD3 tube, mix well and transfer 50 µL of the STD3 standard to the STD2 tube, mix well and transfer 50 µL of the STD2 standard to the STD1 tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Standard Concentration (pg/ml)	Volume of Deionized Water to Add (mL)	Volume of Standard to Add
STD7	250	0

Standard Concentration (pg/ml)	Volume of Assay Buffer to Add (mL)	Volume of Standard to Add
STD6	200	50 mL of STD7
STD5	200	50 mL of STD6

STD4	200	50 mL of STD5
STD3	200	50 mL of STD4
STD2	200	50 mL of STD3
STD1	200	50 mL of STD2

Reconstituted STD6 STD5 STD4 STD3 STD2 STD1 Standard (STD7)

The serial dilutions result in the following concentrations of standards.

Standard Tube #	CRP (pg/ml)	SAA (pg/ml)	SAP (pg/ml)
STD1	3.2	16.0	16.0
STD2	16.0	80.0	80.0
STD3	80.0	400.0	400.0
STD4	400.0	2,000	2,000
STD5	2,000	10,000	10,000
STD6	10,000	50,000	50,000
STD7	50,000	250,000	250,000

IMMUNOASSAY PROCEDURE

 Allow all reagents to warm to room temperature (20-25°C) before use in the assay.

 Run the standards, controls, and samples 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 Wash 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 Wash Buffer by vacuum. Blot excess Wash Buffer from the bottom of the plate with an absorbent pad or paper towels.

3. Add 25 µL of each Standard or Control into the appropriate wells. Add 25 mL Assay Buffer to the 0 pg/mL standard (Background).

4. Add 25 µL of Assay Buffer to the sample wells.

5. Add 25 µL of the Assay Buffer solution to the background, appropriate standards, and control wells.

6. Add 25 µL of Sample into the appropriate wells.

7. 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.)

8. 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 1 hour at room temperature (20-25°C).

9. Gently remove fluid by vacuum.

10. Wash plate 2 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 into each well. **(Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)**

12. Seal, cover with lid, and incubate with agitation on a plate shaker for 30 minutes. **DO NOT**

VACUUM AFTER INCUBATION.

13. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.

14. Seal, cover with lid and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).

15. Gently remove all contents by vacuum.

16. Wash plate 2 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.

17. Add 100 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.

18. Run plate on Luminex 100™ IS.

19. Save and analyze the data using Bio-Plex Manager software.

EQUIPMENT SETTINGS

Events: 50, per bead region Sample Size: 50 µL

Gate Settings 4335 to 10,000

Time Out 60 seconds

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.

NOTES:

Procedure- Quick Reference

Step 2.