Lminex Milliplex Soluble Cytokine Receptor 13-plex

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Abstract

Lminex Milliplex Soluble Cytokine Receptor 13-plex manufacturer's protocol

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Protocol

Step 1.
PREPARATION OF SAMPLES/REAGENTS FOR IMMUNOASSAY

Step 2.
Preparation of Serum/Plasma Thaw Time: Thaw the samples completely on ice, mix well by vortexing and centrifuge (10,000 rpm, 10 minutes, 4°C) prior to use in the assay to remove particulates. Serum/Plasma samples from normal subjects should be diluted 1:5 using the Serum Matrix provided in the kit as the sample diluent (20 µL sample mixed with 80 µL Serum Matrix). If samples require dilution beyond 1:5, continue to use the Serum Matrix as the sample diluent.

Step 3.
Preparation of Antibody-Immobilized Beads For individual vials of beads, sonicate each antibody-bead vial for 30 seconds then vortex for 1 minute. Add 60 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 13 antibody-immobilized beads, add 60 µL from each of the 13bead sets to the Mixing Bottle. Then add 2.22 mL Bead Diluent.

Step 4.
Preparation of Quality Controls Reconstitution Time: Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µLdeionized 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.

Step 5.
Preparation of Wash Buffer Bring the 10X Wash Buffer to room temperature and mix to bring all salts intosolution. Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water. Storeunused portion at 2-8°C for up to one month.

Step 6.
Preparation of Serum Matrix Reconstitution Time: Add 5.0 mL Assay Buffer to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 10 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at £ -20°C for up to one month.

Step 7.
Preparation of Human Soluble Cytokine Receptor Standard Reconstitution Time: 1.) Prior to use, reconstitute the Human Soluble Cytokine Receptor Panel Standard with 250 µL deionized water to

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

Step 8.
) Preparation of Working Standards. Label six polypropylene microfuge tubes STD6, STD5, STD4, STD3, STD2, and STD1.Add 150 µL of Assay Buffer to each of the six tubes.-Prepare serial dilutions by adding 50 µL of 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 and mix well, transfer 50 µL of the STD2 standard to the STD1 tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.StandardVolume of Deionized Water to Add (mL)Volume of Standard to AddOriginal (STD7)2500Standard Concentration (pg/ml)Volume of Assay Buffer to Add (mL)Volume of Standard to AddSTD615050 mL of STD7STD515050 mL of STD6STD415050 mL of STD5STD315050 mL of STD4STD215050 mL of STD3STD115050 mL of STD2Reconstituted STD6 STD5 STD4 STD3 STD2 STD1Standard(STD7)After dilution, each tube has the following concentrations for each analyte:Standard Tube #sIL-4R, sIL-6R, sRAGE, sTNF-R1, sTNF-R2 (pg/ml)sCD30, sGP130, sIL-1R1 (pg/ml)sEGFR, sIL-1RII, sVEGF-R1, sVEGF-R2, sVEGF-R3 (pg/ml)1

Step 9.
2

Step 10.
41222

Step 11.
8

Step 12.
74883

Step 13.
3390.61,9534

Step 14.
31,562.57,81353,1256,25031,250612,50025,000125,000750,000100,000500,000IMMUNOASSAY 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.

Step 15.
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).

Step 16.
Remove Wash Buffer by vacuum. Blot excess Wash Buffer from the bottom of the plate with an absorbent pad or paper towels.10) Add 25 µL of each Standard or Control into the appropriate wells. Add 25 µL Assay Buffer to the 0 pg/mL standard (Background).

Step 17.
Add 25 µL of Assay Buffer to the sample wells.

Step 18.

Add 25 µL of the Serum Matrix solution to the background, appropriate standards, and control wells.

Step 19.
Add 25 µL of Sample into the appropriate wells.

Step 20.
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.)

Step 21.
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 2 hours at room temperature (20-25°C).

Step 22.
Gently remove fluid by vacuum.

Step 23.
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.

Step 24.
Add 25 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)

Step 25.
Seal, cover with lid, and incubate with agitation on a plate shaker for 1 hour. DO NOT VACUUM AFTER INCUBATION.20) Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.

Step 26.
Seal, cover with lid and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).

Step 27.
Gently remove all contents by vacuum.

Step 28.
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.

Step 29.
Add 150 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.

Step 30.
Run plate on Luminex 100™ IS.

Step 31.
Save and analyze the data using Bio-Plex Manager software.EQUIPMENT SETTINGSEvents: 50, per bead region Sample Size: 100 µLGate Settings 4335 to 10,000Time Out 60 secondsQUALITY CONTROLSThe 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.