Lminex Milliplex Cytokine/Chemokine 17-plex MAG

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Abstract

Luminex Mulliplex Cytokine/Chemokine 17-plex MAG manufacturer's protocol

Citation: Troy Kemp, Ligia Pinto Lminex Milliplex Cytokine/Chemokine 17-plex MAG. **protocols.io** dx.doi.org/10.17504/protocols.io.hvfb63n
Published: 01 Aug 2017

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 shaking on plate shaker for 1 min. at RT (20-25°C) and centrifuge (1,700 xg, 10 minutes, 4°C) prior to use in the assay to remove particulates.

Step 3.

Preparation of Antibody-Immobilized Beads Sonicate each individual antibody-bead vial for 30 seconds; 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. (Note: Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit).Example 1: When using 17 cytokine antibody-immobilized beads, add 60 µL fromeach of the 17 bead sets to the Mixing Bottle. Then add 1.98 mL BeadDiluent.

Step 4.

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.

Step 5.

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.

Step 6.

Preparation of Serum Matrix Reconstitution Time: Add 1.0 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 Cytokine Standard Reconstitution Time: 1.) Prior to use, reconstitute the

Human Cytokine Panel II Standard with 250 µLdeionized water to give 100,000 pg/ml for SDF-1a+b; 50,000 pg/ml for MIP-1d, TPO,IL-20, Eotaxin-3, IL-23; 20,000 pg/ml for ENA-78, 6CKine, LIF, IL-21, IL-33; 10,000pg/ml for MCP-4, Eotaxin-2, TRAIL, SCF, TSLP, IL-28A, IL-16; 5000 pg/ml for MCP-2,CTACK; 2000 pg/ml for I-309; and 1000 pg/ml for BCA-1, TARC. Invert the vialseveral times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10minutes and then transfer the standard to an appropriately labeled polypropylenemicrofuge 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 serial dilutions, the tubes should have the following concentrations for constructingstandard curves.Standard Tube #BCA-1,TARC (pg/ml)I-309 (pg/ml)MCP-2,CTACK (pg/ml)MCP-4,Eotaxin-2,TRAIL,SCF,TSLP, IL-28A, IL-16 (pg/ml)ENA-78, 6Ckine, LIF, IL-21, IL-33 (pg/ml)MIP-1d, Eotaxin-3, IL-23, TPO, IL-20 (pg/ml)SDF-1a+b (pg/ml)10.240.49

Step 9.
2

Step 10.
4

Step 11.
9

Step 12.
2

Step 13.
420.98

Step 14.

Step 15.
9

Step 16.
8

Step 17.
5

Step 18.
8

Step 19.
73

Step 20.

9

Step 21.
8

Step 22.
5

Step 23.
1

Step 24.
1

Step 25.
3390.64

Step 26.
6

Step 27.
3

Step 28.
1

Step 29.
3

Step 30.
5

Step 31.
31,562.55

Step 32.
5125

Step 33.
56251,2503,1256,25062505001,2502,5005,00012,50025,00071,0002,0005,00010,00020,00050,000100,000IMMUNOASSAY 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.

Step 34.
Prewet the plate by pipetting 200 µL of Wash Buffer into each well of the MAG Plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).

Step 35.
Decant Wash Buffer and remove residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.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 36.
Add 25 µL of Assay Buffer to the sample wells.

Step 37.
Add 25 µL of the Serum Matrix solution to the background, appropriate standards, and control wells.

Step 38.
Add 25 µL of Sample into the appropriate wells.

Step 39.
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. Due to the composition of magnetic beads, you may notice a slight color in the bead solution. This does not affect the performance of the beads or the kit).

Step 40.
Seal the plate with a plate sealer, cover it with the lid. Wrap a rubber band around the plate, lid, and shaker platform and incubate with agitation on a plate shaker 2 hours at room temperature (20-25°C).

Step 41.
Gently remove fluid by aspiration. Do not invert plate.

Step 42.
Wash plate 2 times with 200 µL/well of Wash Buffer, removing Wash Buffer by aspiration between each wash. To avoid washing/aspiration related bead loss, allow approximately 60 seconds between dispensing of the Wash Buffer and subsequent aspiration.

Step 43.
Add 25 µL of Detection Antibodies into each well (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)

Step 44.
Seal, cover with lid, and incubate with agitation on a plate shaker for 1 hour at room temperature (20-25°C). DO NOT ASPIRATE AFTER INCUBATION.20) Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.

Step 45.
Seal, cover with lid and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).

Step 46.
Gently remove all contents by aspiration. Do not invert plate.

Step 47.
Wash plate 2 times with 200 µL/well of Wash Buffer, removing Wash Buffer by aspiration between each wash. To avoid washing/aspiration related bead loss, allow approximately 60 seconds between dispensing of the Wash Buffer and subsequent aspiration.

Step 48.
Add 150 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.

Step 49.
Run plate on Luminex 100™ IS.

Step 50.
Save and analyze the data using Bio-Plex Manager software.EQUIPMENT SETTINGSEvents: 50, per bead region Sample Size: 100 µLGate Settings 5000 to 25,000Time 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.

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Step 51.