Luminex Milliplex Cytokine/Chemokine 15-plex MAG

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Abstract

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. If samples are in 96 well plates, then 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: When using 15 antibody-immobilized beads, add 60 µL from each of the 15 bead sets to the Mixing Bottle. Then add 2.1 mL Bead Diluent.

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 deionized water 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 Standard with 250 µL deionized water to give a 10,000 pg/mL concentration of standard for all analytes. 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 will be used as the 10,000 pg/mL standard;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 2000, 400, 80, 16, 3.2, and 0.64 pg/mL.Add 200 µL of Assay Buffer to each of the six tubes.-Prepare serial dilutions by adding 50 µL of the 10,000 pg/mL reconstituted standard to the 2000 pg/mL tube, mix well and transfer 50 µL of the 2000 pg/mL standard to the 400 pg/mL tube, mix well and transfer 50 µL of the 400 pg/mL standard to the 80 pg/mL tube, mix well and transfer 50 µL of the 80 pg/mL standard to 16 pg/mL tube, mix well and transfer 50 µL of the 16 pg/mL standard to the 3.2 pg/mL tube and mix well, transfer 50 µL of the 3.2 pg/mL standard to the 0.64 pg/mL 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 Add10,0002500Standard Concentration (pg/ml)Volume of Assay Buffer to Add (mL)Volume of Standard to Add200020050 mL of 10,000 pg/mL40020050 mL of 2000 pg/mL8020050 mL of 400 pg/mL1620050 mL of 80 pg/mL

Step 9.
220050 mL of 16 pg/mL0.6420050 mL of 3.2 pg/mLReconstituted 2000 pg/mL 400 pg/mL 80 pg/mL 16 pg/mL 3.2 pg/mL 0.64 pg/mLStandard10,000 pg/mlMMUNOASSAY 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 10.
Prewet 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 11.
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 12.
Add 25 µL of Assay Buffer to the sample wells.

Step 13.
Add 25 µL of the Serum Matrix solution to the background, appropriate standards, and control wells.

Step 14.
Add 25 µL of Sample into the appropriate wells.

Step 15.
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 16.
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 (16-18 hrs.) at 4°C (use cold room).

Step 17.
Gently remove fluid by aspiration. Do not invert plate.

Step 18.
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 19.
Add 25 µL of Detection Antibodies into each well (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)

Step 20.
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 21.
Seal, cover with lid and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).

Step 22.
Gently remove all contents by aspiration. Do not invert plate.

Step 23.
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 24.
Add 150 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.

Step 25.
Run plate on Luminex 100™ IS.

Step 26.
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.NOTES:Legend: A- Hemolyzed sample; B- Lipemic sample; C- Sample missing; D- Clogged Filter well; E- Low bead count; F- >30% Bead aggregation; G- Instrument repeatProcedure-Quick Reference