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Working

U Mass - Cytokines Panel II - multiplex

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Mouse Metabolic Phenotyping Centers

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ABSTRACT

Summary:

This experiment provides the quantification of multiple cytokines and chemokines using multiplexed-Luminex technology based on beads containing specific antibodies. Serum cytokine levels reflect chronic or acute inflammation, and circulating cytokines and chemokines are altered in obesity.

Cytokines Panel II include IL-16 (interleukin-16), IL-17E/IL-25, IL-21, IL-22, IL-28B, EPO (erythropoietin), Exodus-2 (CCL-21), Fractalkine (CX3CL1), MCP-5 (monocyte chemotactic protein-5; CCL-12), MIP-3α (macrophage inflammatory protein 3-alpha; CCL-20), MIP-3β(macrophage inflammatory protein 3-beta; CCL-19), and TARC (thymus and activation- regulated; CCL-17).

A service can be requested for all or any combination of listed cytokines/chemokines for customized multiplexed Luminex assay.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=191&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Mouse Cytokine & Chemokine Standard	MXM8070	Millipore		
Mouse Cytokine & Chemokine Standard	MXM8070-2	Millipore		
Mouse Cytokine Quality Controls 1 & 2	MXM6070	Millipore		
Mouse Cytokine Quality Controls 1 & 2	MXM6070-2	Millipore		
Serum Matrix (contains 0.8% NaN3)	MXMSM	Millipore		
96 Well Plate & sealers		Millipore		
Assay Buffer	L-AB	Millipore		
10X Wash Buffer(0.05% Proclin)	L0WB	Millipore		
Mouse Cytokine Panel II Detection Antibodies	MXM1070-1, MXM1070-2, MXM1070-3	Millipore		
Streptavidin- Phycoerythrin	L-SAPE3, L-SAPE4, L-SAPE10	Millipore		
Mixing Bottle		Millipore		

MATERIALS TEXT

Additional Items

1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
2. Multichannel Pipettes capable of delivering 5 µL to 50 µL or 25 µL to 200 µL
3. Reagent Reservoirs

4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Aluminum Foil
7. Absorbent Pads
8. Laboratory Vortex Mixer
9. Sonicator (Branson Ultrasonic Cleaner Model # B200 or equivalent)
10. Titer Plate Shaker (Lab-Line Instruments Model #4625 or equivalent)
11. BioRad Bioplex 200
12. Bioplex 2 Pro Automatic Plate Washer

Reagent Preparation:

Reagent 1:
Preparation of Antibody-Immobilized Beads:
1. If pre-mixed beads are used, sonicate the pre-mixed bead bottle for 30 seconds, and vortex for 1 minute before use.

2. For individual vials of beads, sonicate each antibody-bead vial for 30 seconds, and vortex for 1 minute.

3. Add 60 µL from each antibody bead vial to the Mixing Bottle, and bring final volume to 3.0 mL with Assay Buffer.

4. Vortex the mixed beads well.

5. Unused portion may be stored at 2~8°C for up to 1 month. 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.

Reagent 2:
Preparation of Quality Controls:
1. Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL deionized water.

2. Invert the vial several times to mix and vortex.

3. Allow the vial to sit for 5~10 minutes, vortex, and then transfer the controls to appropriately labeled polypropylene microfuge tubes.

4. Unused portion may be stored at ≤ -20°C for up to 1 month.

Reagent 3:
Preparation of Wash Buffer:
1. Bring the 10x Wash Buffer to room temperature and mix to bring all salts into solution.

2. Dilute 60 mL of 10x Wash Buffer (2 bottles) with 540 mL of deionized water.

3. Store unused portion at 2~8°C for up to 1 month.

Reagent 4:
Preparation of Serum Matrix:
1. This step is required for serum or plasma samples only.

2. Add 2.0 mL of Assay Buffer to the bottle containing lyophilized Serum Matrix, and mix well.

3. Allow at least 10 minutes for complete reconstitution.

4. Leftover reconstituted Serum Matrix can be stored at ≤ -20°C for up to 1 month.

Reagent 5:
Preparation of Mouse Cytokine Standard:
1. Prior to use, reconstitute the Mouse Cytokine Standard with 250 µL deionized water to give a 10,000 pg/mL concentration of standard for all analytes.

2. Invert the vial several times to mix, and vortex the vial for 10 seconds.

3. Allow the vial to sit for 5~10 minutes, and transfer the standard to an appropriately labeled polypropylene microfuge tube.

4. This will be used as a 10,000 pg/mL standard. The unused portion may be stored at ≤ -20°C for up to 1 month.

Reagent 6:
Preparation of Working Standards:
1. Label 5 polypropylene microfuge tubes: 2,000, 400, 80, 16, and 3.2 pg/mL.

2. Add 200 µL of Assay Buffer to each of the 5 tubes.

3. Prepare serial dilutions by adding 50 µL of the 10,000 pg/mL reconstituted standard to the 2,000 pg/mL tube, and mix well.

4. Transfer 50 µL of the 2,000 pg/mL standard to the 400 pg/mL tube, and mix well.

5. Transfer 50 µL of the 400 pg/mL standard to the 80 pg/mL tube, and mix well.

6. Transfer 50 µL of the 80 pg/mL standard to 16 pg/mL tube, and mix well.

7. Transfer 50 µL of the 16 pg/mL standard to the 3.2 pg/mL tube, and mix well.

8. The 0 pg/mL standard (Background) will be the Assay Buffer.

BEFORE STARTING

Notes:

√ A maximum of 25 µL per well of diluted serum or plasma samples can be used. Tissue culture or other media samples may also be used.

√ All samples must be stored in polypropylene tubes. Do not store samples using glass tubes.

√ Avoid debris, lipids, and cells when using samples with gross hemolysis or lipemia.

√ Care must be given when using heparin as an anticoagulant since an excess heparin may provide false positive values. In general, do not use more than 10 IU heparin per mL of blood samples collected.

√ When preparing tissue or cell lysates, use non-detergent containing lysis buffers since detergents have an adverse effects on the Luminex Assay.

1 Add 200 µL of Wash Buffer into each well of the plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).

2 Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.

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

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

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

6 When assaying serum or plasma samples, use the Serum Matrix provided with the kit.
When assaying tissue culture or other supernatant, use proper control culture medium as the matrix solution.

7 Add 25 µL of Sample (diluted one part serum or plasma to one part Assay Buffer) into the appropriate wells.

8 Vortex Mixing Bottle and add 25 µL of the mixed or premixed beads to each well. During addition of Beads, shake bead bottle intermittently to avoid settling.

9 Seal the plate with a plate sealer. Wrap the plate with foil and incubate with agitation on a plate shaker overnight (18~20 hours) at 4°C.

10 Allow reagents and assay plate to come to room temperature. Gently remove well contents and wash plate 2x following the instructions listed in the Plate Washing section.

11 Add 25 µL of Detection Antibodies into each well. Allow the Detection Antibodies to warm to room temperature prior to addition.

12 Seal, cover with foil, and incubate with agitation on a plate shaker for 1 hour at room temperature (20~25°C). Do not aspirate after incubation.

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

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

15 Gently remove well contents and wash plate 2x following instructions listed in the Plate Washing section.

16 Add 150 µL of Sheath Fluid to all wells, and resuspend the beads on a plate shaker for 5 minutes.

17 Run plate on Bioplex 200, and set parameters for 125 µl and 75 beads per bead set.

18 Save and analyze the Median Fluorescent Intensity (MFI) data using a 5-parameter logistic or spline curve-fitting method for calculating cytokine/chemokines concentrations in samples. For diluted samples, multiply the calculated concentration by the dilution factor.

19 Plate Washing:
a) Let plate “soak” on magnet for 60 seconds to allow complete settling of the magnetic beads.

b) Remove well contents by aspiration.

c) Wash plate with 200 µL/well of Wash Buffer

d) Allow beads to soak for 60 seconds, and remove Wash Buffer by aspiration after each wash.

e) Repeat wash steps as recommended in the Assay Procedure.

f) If using the recommended plate washer for magnetic beads (Bio-Tek ELx405), follow the instructed equipment settings.