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UC Davis - Luminex/Multiplex V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.56wg9fe

Mouse Metabolic Phenotyping Centers
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ABSTRACT

Summary:

This core will provide services for quantification and comparison in animal models for hormone measurements; kidney function analytes; lipid metabolism analytes; liver function analytes; miscellaneous protein analytes; and immune function including cytokines/chemokines. The Luminex assay enables the quantification of all or any combination of analytes for which there are assay beads in tissue/cell lysate, tissue culture supernatant samples and plasma/serum. For example, cytokines and chemokines are peptides, which act as regulators in normal and pathological conditions, effecting interactions between cells as well as regulating processes occurring in the extracellular environment.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=116&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Human Cytokine/Chemokine standard – lyophilized	MXH8060 OR MXH8060-2	Millipore
Human Cytokine Quality Controls 1 and 2 - lyophilized	MHX6060 OR MXH6060-2	Millipore
Serum Matrix- lyophilized	MXHSM	Millipore
Set of one 96-well filter plate with 2 plate sealers	MX-PLATE	Millipore
96 well plate stand	MX-STAND	Millipore
Assay Buffer 30 mls	L-AB	Millipore
10 x Wash buffer 30 mls	L-WB	Millipore
Human Cytokine Detection Antibodies 3.2 mls	MXH1060-1,2,3,or 4	Millipore
Streptavidin-Phycoerythrin 3.2 mls	*L-SAPE 9,3,10 OR 11	Millipore
Bead Diluent 3.5 mls	LBD	Millipore
Mixing Bottle – 1 bottle		Millipore
Human Cytokine/Chemokine Antibody Immobilized Premixed beads. Example given premixed 14-plex beads	MXHPMX14	Millipore
Sheath Fluid- Bio-plex	171-000055	BioRad Sciences
Calibrator Beads-Bio-plex 10 mls	171-203060	BioRad Sciences
Validation Beads – Bio-plex	171-203001	BioRad Sciences
Adjustable Pipettes with tips 25-1000 uls		Rainin

NAME	CATALOG #	VENDOR
Multichannel Pipettes 5 25 50 200 uls		Rainin
Reagent Reservoirs		Fisher Scientific
Polypropylene Microfuge Tubes		
Aluminum Foil		
Rubber Bands		
Absorbent Pads or paper towels		
Laboratory Vortex Mixer		
Sonicator- Ultrasonic Cleaner or equivalent	Model B200 or equivalent	Branson
Titer Plate Shaker	Model 4625 or equivalent	Lab-Line Instrument
Vacuum Manifold Filtration Unit	MSVMHTS00 or equivalent	Millipore
Bio-Plex- 200 (or Luminex 200 by Luminex Corporation)	Bio-Plex 200 System	BioRad Sciences
Vacuum Pump for use with Vacuum Manifold	WP6111560 or equivalent	Millipore

MATERIALS TEXT

*L-SAPE 9,3,10 OR 11 depending on detection antibody

Note:
Bio-Rad Laboratories, RRID:SCR_008426
Fisher Scientific, RRID:SCR_008452

Reagent Preparation:

1. Preparation of antibody – Immobilized beads
a. For individual beads, sonicate each antibody-bead vial for 30 seconds.
b. For pre-mixed beads, sonicate each bottle for 30 seconds.
c. Vortex each antibody-bead vial for one minute.
d. For pre-mixed beads, vortex bottle for one minute.
e. For individual beads, add 60 ul from each individual antibody-bead vial to the Mixing Bottle and bring final volume to 3.0 ml with bead diluent.
f. Vortex the mixed beads/bead diluent well.

2. Preparation of Quality Controls
a. Before use, reconstitute Quality Control 1 with 250 µl of deionized water.
b. Before use, reconstitute Quality Control 2 with 250 ul of deionized water.
c. Invert Quality Control 1 and Quality Control 2 vials several times.
d. Vortex gently Quality Control 1 and Quality Control 2 vials.
e. Allow both vials to sit for 10 minutes at room temperature.
f. Transfer Quality Control 1 and Quality Control 2 contents to appropriately labeled polypropylene microfuge tubes.

3. Preparation of Wash Buffer
a. Bring the 10X Wash Buffer to room temperature.
b. Dilute 30 ml of 10X Wash Buffer with 270 ml of deionized water.
c. Wash Buffer should be room temperature before use.
d. Wash Buffer can be stored at 2-8 degrees C for up to one month.

4. Preparation of Serum Matrix (required for serum or plasma samples only).
a. Add 1.0 ml of deionized water to the bottle containing lyophilized Serum Matrix.
b. Mix well.
c. Allow Serum Matrix to sit 10 minutes for complete reconstitution.

5. Preparation of Human Cytokine Standard

a. Reconstitute the Human Cytokine Standard with 250 ul of deionized water.
b. Reconstituted standard concentration will be 10,000 pg/ml.
c. Invert the vial several times to mix.
d. Vortex the vial for 10 seconds.
e. Allow the vial to sit for 5-10 minutes at room temperature.
f. Transfer contents to an appropriately labeled polypropylene microfuge tube.
g. The vial should be at room temperature before use.

6. Preparation of Working Standards

a. Label five polypropylene microfuge tubes, 2000, 400, 80, 16, and 3.2 pg/ml.
b. Transfer 200 ul of Assay Buffer to each of the five tubes.

Prepare serial dilutions of standards.

♣ Transfer 50 ul of the 10,000 pg/ml tube to the 2000 pg/ml tube.

♣ Mix the tube gently.

♣ Transfer 50 ul of the 2,000 pg/ml tube to the 400 pg/ml tube.

♣ Mix the tube gently.

♣ Transfer 50 ul of the 400 pg/ml tube to the 80 pg/ml tube.

♣ Mix the tube gently.

♣ Transfer 50 ul of the 80 pg/ml tube to the 16 pg/ml tube.

♣ Mix the tube gently.

♣ Transfer 50 ul of the 16 pg/ml tube to the 3.2 pg/ml tube.

♣ Mix the tube gently.

7. Vortex all Reagents before adding to plate.

SAFETY WARNINGS

WARNING:

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions established by CDC and OSHA when handling and disposing of infectious agents.

Sodium azide or Proclin has been added to some reagents as a preservative. When disposing, flush with a large volume of water to prevent azide build up.

BEFORE STARTING

IMPORTANT: All plasma, serum, tissue supernatants must be centrifuged for 10 minutes at 12,000 rpms to clarify the biological fluid. Avoid multiple freeze thaws >2. All reagents must be prepared prior to step one and be at room temperature, beads must be sonicated and vortexed, standards and QC controls reconstituted and after 10 minutes and transferred to a polypropylene tube. Always place filter plate on the plate stand except when blotting post wash. Thorough blotting post wash and pre-incubation is critical to avoid leakage due to capillary action. During the incubation step, the filter plate must be covered with a plate sealer and wrapped in

foil due to light sensitivity of the beads. Never invert the plate. Vacuum suction <100mmHg.

1 Pre-wet the filter plate with 200 ul assay buffer, agitate, incubate for 10 minutes RT and vacuum.

2 Add 25 ul of standards, controls, assay buffer, samples, matrix serum and beads to appropriate wells on the filter plate.

3 Place filter plate on plate stand, seal, wrap in foil and incubate overnight while agitating on a Titer Plate Shaker at 4 degrees C (16-18 hours).

4 Remove fluid from the filter plate by vacuum and wash. Bolt well.

5 Add 25 ul of detection antibody to each well, place filter plate on plate stand, seal, wrap in foil, agitate on a Titer Plate Shaker for 1 hour at RT.

6 Without removing detection antibody, add 25 ul of Streptavidin- Phycoerythrin to each well.
Place filter plate on plate stand, seal, wrap in foil, agitate on the Titer Plate Shaker. Incubate for 30 mins RT.

7 Remove the fluid by vacuum and wash the plate. Bolt well.

8 Add 150 uls of Sheath Fluid to each well, place on plate stand, seal, wrap in foil, agitate on the Titer Plate Shaker at RT for 5 minutes.

9 Set up protocol on the Bio-Plex 200: add analytes and regions, standards, quality controls and sample dilutions to Bio-Plex 6.0 Manager software.

10 Run plate on Bio-Plex (Luminex) 200.

11 Save and analyze the median fluorescent intensity data using a 5-parameter logistic or spline curve fitting method for calculating cytokine/chemokines concentrations in samples in pg/ml.