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U Mass - Leptin

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Protocol status: Working

We use this protocol and it's working

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Abstract

Summary:

This experiment provides the quantification of multiple hormones using multiplexed-Luminex technology based on beads containing specific antibodies. Sample matrices include plasma, serum, tissue culture supernatants, and tissue or cell lysates. Leptin is an adipocyte-derived hormone (adipokine) that regulates feeding behavior (hunger/satiety). Serum leptin levels are altered in obesity, insulin resistance, and type 2 diabetes.



Materials

MATERIALS

- ✕ Mouse Metabolic Hormone Standard **Merck Millipore (EMD Millipore) Catalog #MXM8070**
- ✕ Mouse Hormone Quality Controls 1 & 2 **Merck Millipore (EMD Millipore) Catalog #MXM6070**
- ✕ Mouse Hormone Quality Controls 1 & 2 **Merck Millipore (EMD Millipore) Catalog #MXM6070-2**
- ✕ Serum Matrix (contains 0.8% NaN₃) **Merck Millipore (EMD Millipore) Catalog #MXMSM**
- ✕ 96 Well Plate & sealers **Merck Millipore (EMD Millipore)**
- ✕ Assay Buffer **Merck Millipore (EMD Millipore) Catalog #L-AB**
- ✕ 10X Wash Buffer (0.05% Proclin) **Merck Millipore (EMD Millipore) Catalog #L0WB**
- ✕ Mouse Hormone Detection Antibodies **Merck Millipore (EMD Millipore) Catalog #MXM1070-1, MXM1070-2, MXM1070-3**
- ✕ Streptavidin- Phycoerythrin **Merck Millipore (EMD Millipore) Catalog #L-SAPE3, L-SAPE4, L-SAPE10**
- ✕ Mixing Bottle **Merck Millipore (EMD Millipore)**

Additional Items

1. Adjustable Pipettes with Tips capable of delivering 25 μ L to 1000 μ L
2. Multichannel Pipettes capable of delivering 5 μ L to 50 μ L or 25 μ L to 200 μ L
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. Sonicate each antibody-bead vial for 30 seconds, and vortex for 1 minute.
2. Add 150 μ L from each antibody bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Bead Diluent.
3. Vortex the mixed beads well.

4. 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 $\leq -20^{\circ}\text{C}$ 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 30 mL of 10x Wash Buffer with 270 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 1.0 mL of deionized water 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 $\leq -20^{\circ}\text{C}$ for up to 1 month.

Reagent 5:

Preparation of Mouse Metabolic Hormone Standards:

1. Prior to use, reconstitute the Mouse Metabolic Hormone Standard with 250 µL of deionized Water.
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, vortex, and then transfer the standard to appropriately labeled polypropylene microfuge tube.



4. This will be used as Standard 1.
5. Label 6 polypropylene microfuge tubes, #2~#7, and add 100 μ L of Assay Buffer to each tube.
6. Make 3-fold serial dilutions from the Stock Standard (Standard 1) by adding 50 μ L of Standard 1 to Standard 2.
7. Vortex to mix, and with a new tip transfer 50 μ L of Standard 2 to Standard 3.
8. Repeat for Standard 4-7.

Troubleshooting

Before start

Notes:

- ✓ A maximum of 10 μ L per well of diluted serum or plasma samples can be used. Tissue culture or other media samples can 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 μ L of Assay 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 Assay Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times.
- 3 Add 10 μ L of appropriate matrix solution to the background, standards, and control wells.
- 4 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.
- 5 Add 10 μ L of Assay Buffer to the background (0 pg/ml standard) and sample wells.
- 6 Add 10 μ L of each Standard or Control into the appropriate wells.
- 7 Add 10 μ L of sample into the appropriate wells.
- 8 Vortex Mixing Bottle and add 25 μ L 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 3x following the instructions listed in the Plate Washing section.
- 11 Add 50 μ L 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 30 minutes at room temperature (20~25°C). Do not aspirate after incubation.
- 13 Add 50 μ L Streptavidin-Phycoerythrin to each well containing the 50 μ L 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 3x following instructions listed in the Plate Washing section.
- 16 Add 150 μ L 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 μ l 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 μ L/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.