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Working

U Mass - Adiponectin

Jason Kim[1]

[1]University of Massachusetts

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Mouse Metabolic Phenotyping Centers

Tech. support email: **info@mmpc.org**

Lili Liang

ABSTRACT

Summary:

The adiponectin enzyme-linked immunosorbent assay (ELISA) is a standard immunoassay for the detection of total adiponectin levels in serum, adipocyte extracts, and cell culture media samples. This experiment uses a 96-well plate coated with anti-adiponectin antibodies and developed with a secondary anti-adiponectin antibody linked to horseradish peroxidase. The ELISA is developed with 3,3',5,5'-Tetramethylbenzidine (TMB), a chromogenic substrate and read on an automated plate spectrophotometer at 450 nm. Adiponectin is an adipocyte-derived hormone (adipokine) that regulates insulin sensitivity and glucose metabolism.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=165&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Adhesive Plate Sealer	EZMADP-60K(kit)	Millipore Sigma		AB_2651034
10X HRP Wash Buffer	EZMADP-60K(kit)	Millipore Sigma		
Mouse Adiponectin Standard	EZMADP-60K(kit)	Millipore Sigma		
Quality Controls (1 & 2)	EZMADP-60K(kit)	Millipore Sigma		
10X Assay Buffer	EZMADP-60K(kit)	Millipore Sigma		
Mouse Adiponectin Detection Antibody	EZMADP-60K(kit)	Millipore Sigma		
Enzyme Solution (Streptavidin-HRP)	EZMADP-60K(kit)	Millipore Sigma		
Substrate (TMB)	EZMADP-60K(kit)	Millipore Sigma		
Stop Solution (0.3M HCl)	EZMADP-60K(kit)	Millipore Sigma		
Adiponectin Microplate (96 wells)	EZMADP-60K(kit)	Millipore Sigma		

MATERIALS TEXT

Note:
EZMADP-60K (kit), Cite this **(Millipore Cat# EZMADP-60K, RRID: AB_2651034)**

Additional Items

1. Pipettes and Pipette Tips: 10µL - 20 µL or 20µL - 100 µL
2. Multi-Channel Pipettes and Pipette Tips: 5 ~ 50 µL and 50 ~ 300 µL
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. Deionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm
7. Orbital Microtiter Plate Shaker

8. Absorbent Paper or Cloth

Reagent Preparation:

Reagent 1:

Mouse Adiponectin Standard Preparation:

1. Use care in opening the lyophilized Standard vial.
2. Using a pipette, reconstitute the Mouse Adiponectin Standard with 1.0 mL distilled or deionized water into the vial to give a concentration prescribed in the analysis sheet.
3. Invert and mix gently.
4. Wait 5 minutes, and mix well.
5. Label seven tubes 1, 2, 3, 4, 5, 6, and 7. Add 0.5 mL Assay Buffer to each of the seven tubes.
6. Prepare serial dilutions by adding 0.5 mL of the reconstituted standard to tube 1, and mix well.
7. Transfer 0.5 mL of tube 1 to tube 2, and mix well.
8. Transfer 0.5 mL of tube 2 to tube 3, and mix well.
9. Transfer 0.5 mL of tube 3 to tube 4, and mix well.
10. Transfer 0.5 mL of tube 4 to tube 5, and mix well.
11. Transfer 0.5 mL of tube 5 to tube 6, and mix well.
12. Transfer 0.5 mL of tube 6 to tube 7, and mix well.

Reagent 2:

Mouse Adiponectin Quality Control 1 and 2 Preparation:

1. Use care in opening the lyophilized Quality Control vials.
2. Using a pipette, reconstitute each of the Mouse Adiponectin Quality Control 1 and Quality Control 2 with 1 mL distilled or deionized water into the vials.
3. Invert and mix gently.
4. Incubate for 5 minutes, and then mix well.

BEFORE STARTING

Sample Preparation:

1. Dilute serum or plasma samples 1:1,000 in 1x Assay Buffer (See Section III, F). Cellular extract and culture media dilutions will vary.
   a) Make Dilution A by adding 10 µL sample to 990 µL Assay Buffer and vortex.
   b) Make Dilution B by adding 100 µL of Dilution A to 900 µL Assay Buffer and vortex.
2. Use Dilution B (1:1,000) for the assay procedure.

Notes:

√ Use freshly prepared serum, plasma, or aliquot, and store samples at –20º C for later use.
√ For long-term storage, keep samples at -70º C. Avoid freeze/thaw cycles.
√ Avoid using samples with gross hemolysis or lipemia.
√ Allow all the reagents to reach room temperature before use

1 Dilute the 10x Wash Buffer concentrate 10-fold by mixing the entire content of each bottle of Wash Buffer with 450 mL deionized water (dilute both bottles with 900 mL deionized water).

2 Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8°C.

3 Assemble the strips in an empty plate holder and wash each well 3x with 300 µl of diluted Wash Buffer per wash.

4 Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, use a gentle wash program for all washing steps described in this protocol.

5 Add 60 µl Assay Buffer to all wells.

6 Add in duplicate 20 µl Assay Buffer to the blank wells.

7 Add in duplicate 20 µl Mouse Adiponectin Standards in the order of ascending concentration to the appropriate wells.

8 Add in duplicate 20 µl QC1 and 20 µl QC2 to the appropriate wells. Add sequentially 20 µl of the unknown samples in duplicate to the remaining wells.

9 Add 20 µl Detection Antibody to all wells. For best result all additions should be completed within 1hour.

10 Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed (400~500 rpm).

11 Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.

12 Wash wells 5x with diluted Wash Buffer, 300 µl per well per wash. Decant and tap firmly after each wash to remove residual buffer.

13 Add 100 µl Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

14 Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.

15 Wash wells 5x with diluted Wash Buffer, 300 µl per well per wash. Decant and tap firmly after each wash to remove residual buffer.

16 Add 100 µl of Substrate Solution to each well, cover plate with sealer, and shake on the plate shaker for approximately 5~20 minutes. Blue color should be formed in wells of Adiponectin standards with intensity proportional to increasing concentrations of Adiponectin.

NOTE: Be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Visually monitor the color development to optimize the incubation time. One can monitor color development using 370 nm filter, if available on the spectrophotometer. When the absorbance is between 1.2 and 1.8 at 370 nm, the stop solution can be added to terminate the color development.

17 Remove sealer and add 100 µl stop solution [**CAUTION: CORROSIVE SOLUTION**] and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification.

18 Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well.

19 Record the difference of absorbance units. The absorbance of highest Adiponectin standard should be approximately 2.2-2.8 or not to exceed the capability of the plate reader used.