MAY 09, 2019

U Mass - Adiponectin

Jason Kim¹

¹University of Massachusetts

Mouse Metabolic Phenotyping Centers
Tech. support email: info@mmpc.org

Lili Liang

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.xm9fk96

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

Protocol Citation: Jason Kim 2019. U Mass - Adiponectin. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.xm9fk96>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: Jan 31, 2019

Last Modified: May 09, 2019

PROTOCOL integer ID:
19873

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.

MATERIALS

MATERIALS

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

Note:

EZMADP-60K (kit), Cite this (**Millipore Cat# EZMADP-60K**, [RRID: AB_2617149](https://rrid.info/RRID/AB_2617149))

Keywords: Adiponectin, enzyme-linked immunosorbent assay (ELISA), insulin sensitivity, glucose metabolism

AB_2651034

Additional Items

1. Pipettes and Pipette Tips: 10 µL - 20 µL or 20 µL - 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 START INSTRUCTIONS

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 μ L sample to 990 μ L Assay Buffer and vortex.
 - b) Make Dilution B by adding 100 μ L of Dilution A to 900 μ L 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 -20° C for later use.
- ✓ For long-term storage, keep samples at -70° 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 $2-8^{\circ}$ C.
- 3 Assemble the strips in an empty plate holder and wash each well 3x with 300 μ L 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 μ L Assay Buffer to all wells.

- 6** Add in duplicate 20 μ l Assay Buffer to the blank wells.
- 7** Add in duplicate 20 μ l Mouse Adiponectin Standards in the order of ascending concentration to the appropriate wells.
- 8** Add in duplicate 20 μ l QC1 and 20 μ l QC2 to the appropriate wells. Add sequentially 20 μ l of the unknown samples in duplicate to the remaining wells.
- 9** Add 20 μ l Detection Antibody to all wells. For best result all additions should be completed within 1 hour.
- 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 μ l per well per wash. Decant and tap firmly after each wash to remove residual buffer.
- 13** Add 100 μ l 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.