ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Mouse Adiponectin ELISA Kit

(Catalog Number: 32010)

For the quantitative determination of mouse adiponectin concentrations in serum, plasma or cell culture supernate samples

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Version 2.0

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INTRODUCTION

Adiponectin, also known as apM1, Acrp30, GBP28 and adipoQ, is a circulating hormone predominantly produced from adipose tissue[1]. Many pharmacological studies demonstrated that this protein possesses potent anti-diabetic, anti-atherogenic and anti-inflammatory functions. Supplementation of adiponectin protein can decrease blood glucose[2], improve insulin sensitivity[3], alleviate fatty liver[4] and prevent atherosclerosis[5]. The protein is post-translationally modified by hydroxylation and glycosylation[6], and forms three different oligomeric complexes in the circulation[7]. Many clinical studies demonstrated that plasma adiponectin is a useful biomarker for metabolic syndrome, nonalcoholic steatohepatitis and certain type of cancers[1]. Decreased circulating levels of plasma adiponectin (hypoadiponectinemia) are associated with increased body mass index (BMI), decreased insulin sensitivity, less favorable plasma lipid profiles, increased levels of inflammatory markers and increased risk for the development of type 2 diabetes, hypertension, and coronary heart diseases. Low adiponectin concentrations were found to be predictive of a future reduction in insulin sensitivity and cardiovascular disorders. Administration of the anti-diabetic drugs thiazolidinediones (TZDs) raises circulating adiponectin levels[8]. In addition, low plasma adiponectin levels are also associated with nonalcoholic steatohepatitis (NASH) and certain types of cancers.

PRINCIPLE OF THE ASSAY

This assay is a sandwich enzyme-linked immunosorbent assay (ELISA) using affinity-purified polyclonal antibodies against mouse adiponectin. The microtiter plate is pre-coated with anti-mouse adiponectin capture antibody. Standards and samples are pipetted into the wells and any mouse adiponectin present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked polyclonal antibody specific for mouse adiponectin is added to the wells. After a final wash step, an HRP substrate solution is added and color develops in proportion to the amount of mouse adiponectin bound initially. The assay is stopped, and the optical density of the wells is determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured mouse adiponectin, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Mouse Adiponectin ELISA kit is designed for quantification of mouse adiponectin in serum, plasma, adipocyte extracts and cell culture media.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter strips (96 wells), coated with anti-mouse adiponectin polyclonal antibody, sealed
2. 10×Wash buffer, 40 mL
3. 5×Assay buffer, 25 mL

4. 100×Detection antibody solution, a polyclonal antibody against mouse adiponectin conjugated with horseradish peroxidase, 0.12 mL
5. Mouse adiponectin standard, 200 ng of recombinant mouse adiponectin, lyophilized
6. Substrate solution, 12 mL, ready for use
7. Stop solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the mouse adiponectin microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 28°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (25 mL) with 100 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 mL) with 360 mL of distilled water or deionized water. The 1×Wash buffer may be stored at 2-8°C for up to one month.

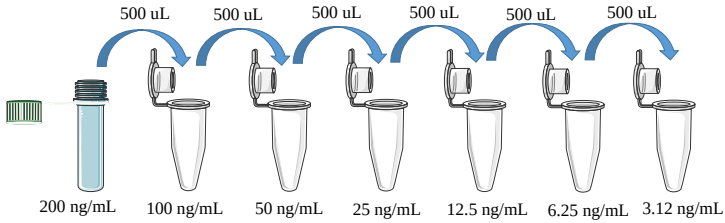
C. 1×Detection antibody solution

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Mouse Adiponectin Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 200 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of

1×Assay buffer to 100, 50, 25, 12.5, 6.25, 3.12 ng/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and frozen at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires a **400-fold** dilution with 1×Assay buffer. Option 1 (One-step dilution)-Add 2 µL of sample to 798 µL of 1×Assay buffer to give a 400-fold diluted sample solution.

Option 2 (Two-step dilution)-Add 10 µL of sample to 190 µL of 1×Assay buffer to give a 20-fold diluted sample solution. Add 20 µL of the 20-fold diluted sample solution to 380 µL of 1×Assay buffer to give a final 400-fold diluted sample solution.

Cellular extract and culture media dilutions will vary and need to be optimized by the user, also use 1×Assay buffer to prepare these samples. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µL of standard or sample per well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µL of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 4 times as described in step 2.
5. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
6. Add 100 µL of Stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Measure absorbance of each well at 450 nm immediately.

CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.

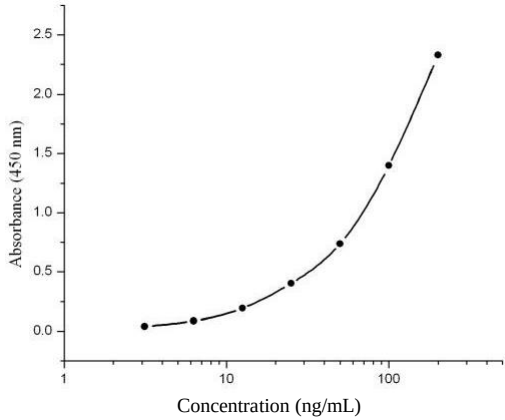
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against mouse adiponectin concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine mouse adiponectin concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each set of sample assay.

Adiponectin (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.064	0
3.12	0.105	0.041
6.25	0.151	0.087
12.5	0.257	0.193
25	0.467	0.403
50	0.801	0.737
100	1.463	1.399
200	2.393	2.329

Mouse adiponectin standard curve (4-parameter)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of mouse adiponectin that can be detected by this assay is 3.12 ng/mL.

B. Specificity

The antibodies used in this assay are specific to mouse adiponectin and do not cross-react with human adiponectin, and other cytokine or hormone molecules.

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8. Maeda N. et al. (2001) *Diabetes.* 50:2094-2099.

SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well 3 times.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well 4 times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation