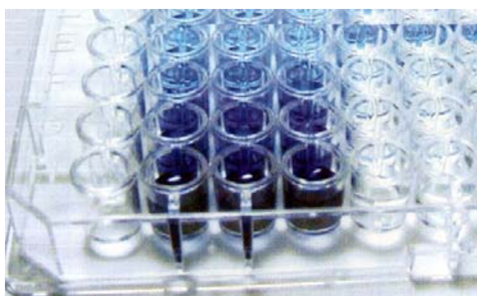
Mouse Adiponectin ELISA Kit

Cat. No. A0322EK



Instruction Manual
Version 1.2.2

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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Introduction

It is becoming clear that adipose tissue is not merely a storage place for excess energy unused but secretes a number of biologically active soluble factors collectively named adipocytokines, thereby regulating glucose and fatty acid metabolism. The gene encoding adiponectin was identified as an adipose tissue-specific gene by screening a subtractive adipocyte cDNA library (1). The gene product was independently identified as gelatin-binding protein-28 (GBP28) (2). The mouse orthologue was isolated by several groups and designated adipocyte complement-related protein of 30kDa (Acrp30), adipocyte most abundant protein-1(APM1), or AdipoQ (3-4). Albeit differently being named it was unequivocally concluded that adiponectin is an adipocyte-specific protein and represents a major serum protein. The full length adiponectin in plasma exists as trimer, hexamer, and multimer whereas extremely low amount of globular domain itself also exists in plasma as trimer (5). Due to its adipocyte-specific expression it was thought that adipo-nectin could be responsible for regulation of adipocyte physiology. In many rodent models, it has been shown that infusion of adiponectin ameliorates hyperglycemia and hyperinsulinemia (6-8). One notable mechanism for decreasing blood sugar is that adiponectin inhibits hepatic glucose production by downregulating the key enzymes involved in gluconeogenesis such as phophoenolpyruvate carboxy-kinase and glucose-6-phosphatase (9). When skeletal muscle cells or a muscle cell line is treated with adiponectin, both glucose uptake and fatty acid oxidation are significantly upregulated (6-8). These studies suggests an unifying conclusion that adiponectin regulates both systemic and hepatic insulin resistance. A direct evidence was shown that administration of adiponectin reduces atherosclerosis in apolipo of

protein E-deficient mice (10). Therefore, measurement serum adiponectin levels gives us an important information on the role of adiponectin in regulation of glucose and/or lipid metabolism.

Assay Principles

This kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of adiponectin in mouse serum, plasma or cell culture supernatants.

Monoclonal antibody specific for mouse adiponectin has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and any adiponectin present is bound by immobilized antibody. Bound adiponectin is captured by purified anti-mouse adiponectin polyclonal antibody. HRP conjugated anti-rabbit IgG is added. After washing, a substrate solution is added. The colors develop in proportion to the bounded adiponectin quantity. The color development is stopped and the intensity of color is measured.

Kit Components

1) Antibody coated 96-well plate, 12 X 8-well strips
2) 5x Wash concentrate, 100 ml
3) 5x Diluent, 50 ml
4) Secondary antibody, 12 ml
5) 100x Detector, 150 μl
6) Standard, recombinant mouse adiponectin expressed by HEK 293 cells, 1 vial, lyophilized
7) QC sample = a positive control of mouse serum adiponectin, 1 vial, lyophilized (For actual concentrations of QC sample, see the 'Certificate of analysis' enclosed.)
8) Substrate I, 6 ml
9) Substrate II, 6 ml
10) Stop solution, 12 ml

Reagents Description

Antibody coated 96-well plate, 12 x 8-well strips, with absorbed monoclonal antibody against mouse adiponectin

5x Wash concentrate, buffered detergent solution, supplied as a 5x concentrate

5 x Diluent, for sample and reagent dilution

1x Secondary antibody, rabbit polyclonal antibody against mouse adiponectin

100x detector, HRP conjugated anti-rabbit IgG

Standard, 16.0 ng, recombinant mouse adiponectin

QC sample, 1 vial each, mouse serum

Substrate I and II, chromogenic reagents

Stop solution, 1 M H_3PO_4

Storage of Reagents

Reagent must be stored at 2-8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Materials Required but not Supplied

Precision single and multi-channel pipettes
Disposable pipette tips
Microtubes or equivalent for preparing dilutions
Disposable plastic containers for preparing working reagents
Reagent reservoirs
Microwell or microstrip plate reader 450 nm
Deionized water

Plasma Collection and Storage

Blood samples for measurement of plasma adiponectin are collected in vacutainer tube and all tubes are centrifuged at 4°C for collection of plasma. These are stored at -80°C until analyses

Assay Procedure

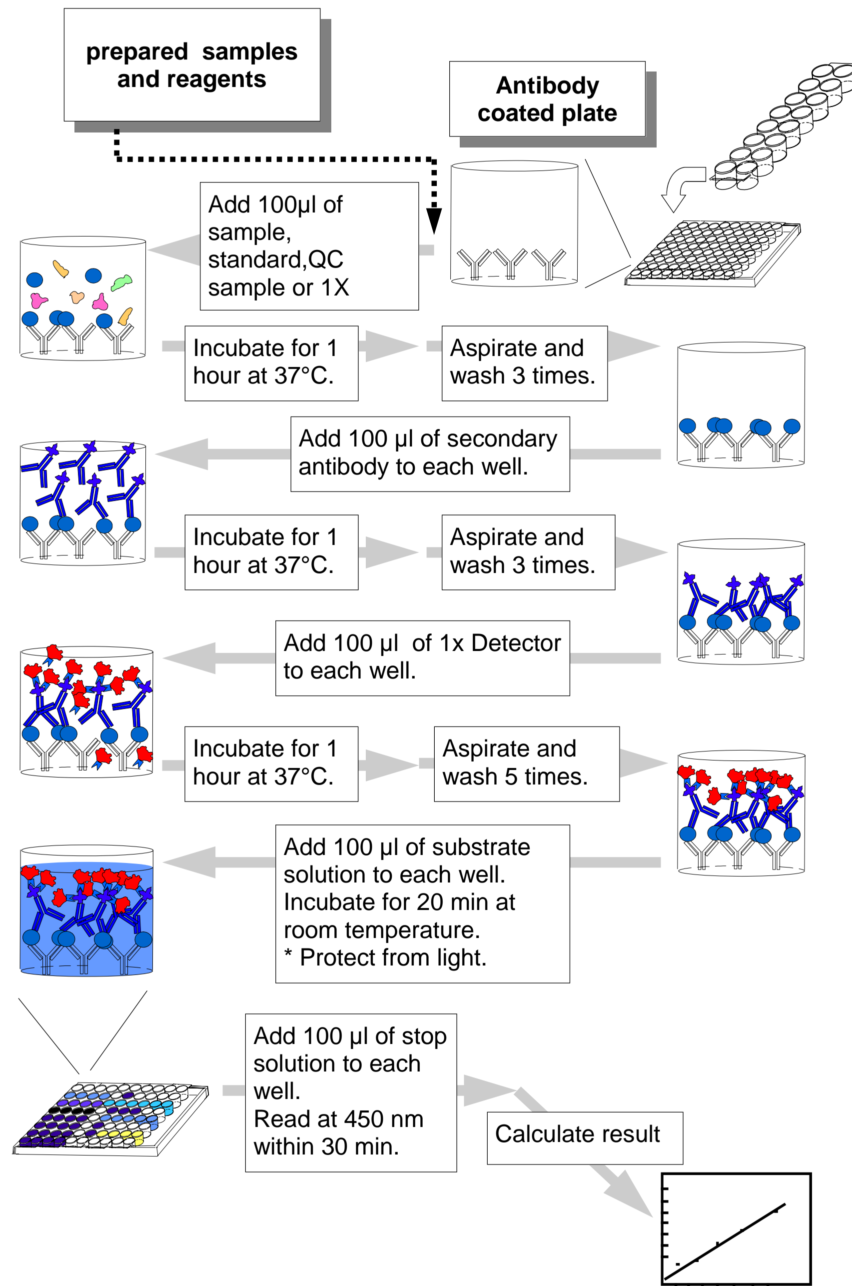
1) Preparation of Working Reagents

1. Allow all samples and kit components to equilibrate to room temperature (20-25°C).
2. Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use (See table below).
   It is recommended that standards and samples be run in duplicate.
3. Prepare **1X Wash Solution**. Dilute 5X Wash Concentrate 1:5 with deionized water (1 part 5X Wash Concentrate with 4 parts deionized water). The diluted 1X Wash Solution is stable for one month at room temperature.
4. Prepare **1X Diluent**. Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).
5. Prepare **1X Detector**. Dilute 100X Detector 1:100 with 1X Diluent (1 part 100X Detector with 99 parts 1X Diluent). Use the 1X Detector within one hour of preparation.
6. Freshly prepare just before use the **Substrate Solution** by adding one part Substrate Ⅰ to one part Substrate Ⅱ.

The amount of working reagents needed for 1 well

Working reagents	Total volume needed	Stock solution added	Dilution solution added	Note
1X Wash Solution	2.8 ml	0.56 ml of 5X Wash Concentrate	2.24 ml of ddH_2O	Stable for 1 month at RT
1X Diluent	2.5 ml	0.5 ml of 5X Diluent	2 ml of ddH_2O	in the case of 10 μl plasma
1X Detector	110 μl	1.1 μl of 100X Detector	108.9 μl of 1X Diluent	Use within 1 hr.
Substrate Solution	110 μl	55 μl of Substrate I	55 μl of Substrate II	Freshly prepared just before use

Flow Chart of Assay Procedure



7. Prepare working aliquots of the Standard as follows :

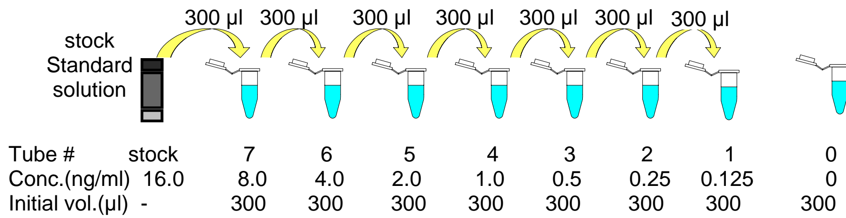
When opening the lyophilized Standard, remove cap gently as the lyophilizate may have become dislodged during shipping. Add 1 ml of deionized water to the Standard vial to make a stock concentration of 16.0 ng/ml. Mix well.

A recommended dilution scheme is as follows :

a) Label 8 microcentrifuge tubes #0-7. Add 300 µl of the 1X Diluent to the microcentrifuge tubes #1~7 and #0, respectively.

b) Add 300 µl of the stock Standard solution to tube #7 and vortex. This is Standard tube #7 with a concentration of 8.0 ng/ml.

c) Standards #6 to #1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube #0.



Tube #	stock	7	6	5	4	3	2	1	0
Conc.(ng/ml)	16.0	8.0	4.0	2.0	1.0	0.5	0.25	0.125	0
Initial vol.(µl)	-	300	300	300	300	300	300	300	300

8. Reconstitute QC sample in 1 ml of deionized water.

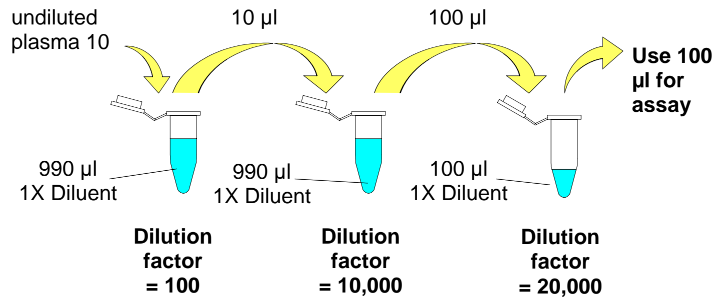
2) Sample preparation

Step 1. Dilute serum 1:100 with 1X Diluent (example, 10 µl serum plus 990 µl 1X Diluent; dilution factor=100) and mix well.

Step 2. Dilute the step 1 1:100 with 1X Diluent (example, 10 µl of step 1 plus and 990 µl 1X Diluent, dilution factor=10,000)

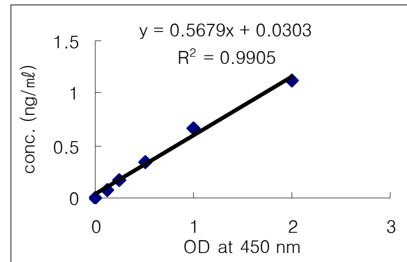
Step 3. Dilute the step 2 1:2 with 1X Diluent (example, 100 µl of 2 step plus 100 µl 1X Diluent; dilution factor= 20,000)

* If samples fall the outside range of assay, a lower or higher dilution may be required.



3) Experiment procedure

1. Remove the appropriate number of microwell strips from the sealed foil pouch.
2. Pipette 100 µl of Standard 0 to 7, the reconstituted QC sample and pre-treated plasma sample into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
3. Incubate at 37°C for 1 hour.
4. Remove the solution and wash 3 times with 250 µl of 1X Wash Solution to each well.
5. Add 100 µl Secondary Antibody to each well.
6. Incubate at 37°C for 1 hour.
7. Remove the solution and wash 3 times with 250 µl of 1X Wash Solution to each well.



8. Add 100µl 1X Detector to each well
9. Incubate at 37°C for 1 hour.
10. Remove the solution and wash 5 times with 250 µl of 1X Wash Solution to each well.
11. Add 100 µl of the Substrate Solution to each well.
12. Incubate at room temperature for 20 min.
    * Protect from light.
13. Using the multi-channel pipette, add 100 µl Stop Solution to each well.
14. Read at 450 nm.
15. Subtract the absorbance of the blank from the readings for each standard and sample.
16. Construct the standard curve by plotting the known concentration (X) of standard versus the absorbance (Y) of standard. A typical linear range is shown between 0.125 ng/ml and 8 ng/ml.
17. Calculate the adiponectin concentrations of samples by interpolation of the regression curve formula.
18. The adiponectin concentrations calculated must be multiplied by dilution factor [see **2) Sample pre-treatment**] to obtain the concentrations of the undiluted samples (Dilution factor of QC sample is 20,000).

Performance Characteristics

1) Sensitivity

The limit of detection: 50 pg/ml

2) Precision

a. Intra-Assay (precision within an assay)

5 samples of known concentration were tested 10 times on one plate to assess intra-assay precision.

Sample	Average (μg/ml)	CV(%)
1	19.16	1.91
2	15.52	4.15
3	23.99	1.44
4	17.62	2.45
5	17.19	3.23

b. Inter-Assay (precision between assays)

5 samples of known concentration were tested 10 times on separated plate to assess inter-assay precision.

Sample	Average (μg/ml)	CV(%)
1	18.01	2.71
2	15.07	6.62
3	18.78	4.19
4	15.95	7.88
5	16.17	4.80

3) Recovery

The recovery of Adiponectin spiked to four different levels in five different mouse serum samples throughout the range of assay was evaluated.

Sample No.	Average recovery (%)	Range (%)
1	102.24	98~105
2	95.95	93~98
3	95.97	91~98
4	94.98	92~97
5	98.03	93~102

4) Specificity

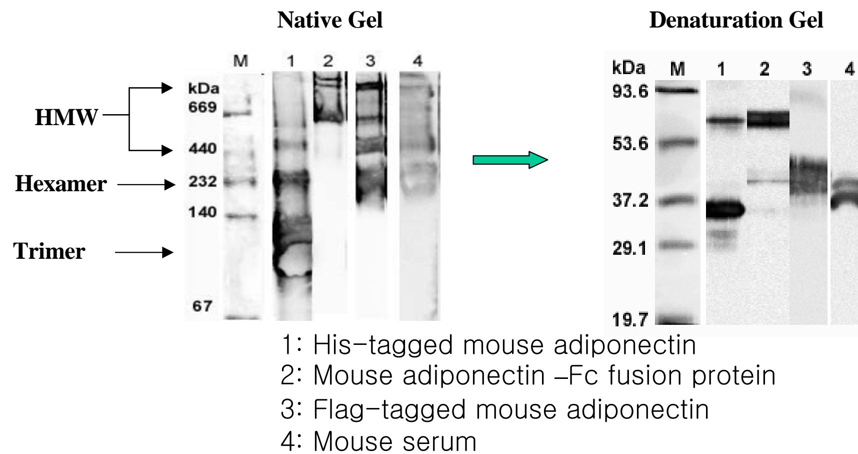
a. No cross reaction with human and rat sera

b. Cross Reactivity

Analyte	Max. Conc. (ng/ml)	Cross Reactivity (%)
Mouse Adiponectin	10	100
Human Adiponectin	10	N. R.
Rat Adiponectin	10	N. R.
Mouse Resistin	100	N. R.
Mouse RELM-β	100	N. R.
Mouse Leptin	100	N. R.
Human TNF-α	100	N. R.

N. R : No Cross-Reactivity

5) Accessibility of the capture antibody to different conformations of native adiponectin



1: His-tagged mouse adiponectin
2: Mouse adiponectin -Fc fusion protein
3: Flag-tagged mouse adiponectin
4: Mouse serum

HMW : High Molecular Weight form

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	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

	1	2	3	4	5	6	7	8	9	10	11	12
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Troubleshooting Guide

Problem	Possible Cause	Solution
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be appropriate for the system.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of detector too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.
	Technique problem	Proper mixing of reagents and wash steps are critical.

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