

Human Adiponectin Competitive ELISA Kit

Cat. No. AC05F2EK



Instruction Manual
Version 2.1.1

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES



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Introduction

Adipocytes express a variety of adipocytokines that function in the homeostatic control of glucose and lipid metabolism. Insulin regulates secretion of many of these adipocytokines in response to changes in energy balance. Adiponectin is a 244-amino acid protein with high structural homology to collagen VIII, collagen V, complement C1q(Ref. 1 and 2), and TNF(Ref.3), which is exclusively and abundantly expressed in white adipose tissue. Plasma adiponectin concentrations have found to be decreased in obesity and/or type-2 diabetes, resulting in the conditions commonly associated with insulin resistance and hyper-insulinemia(Ref. 4-5). Therefore, measurement of the plasma level of adiponectin may be important for understanding diagnosis or prognosis of onset of these diseases.

Assay Principles

This kit is a competitive enzyme-linked immunosorbent assay (ELISA) for quantitative determination of adiponectin in human serum, plasma or various tissue or cell culture supernatants. A polyclonal antibody recognizing native human adiponectin reacts with a series of predetermined recombinant adiponectin standard proteins or serum or plasma under competition in the adiponectin-coated plate. Their relative reactivity is plotted with that of the standard proteins.

Kit Components

- 1) Recombinant human adiponectin coated 96-well plate, 12X 8-well strips
- 2) 5X Wash concentrate, 100 ml
- 3) 5X Diluent, 50 ml
- 4) Antibody, 12 ml
- 5) 100X Detector, 150µl
- 6) Standard, recombinant human adiponectin expressed by HEK 293 cells, 1 vial, lyophilized
- 7) QC sample = a positive control having 7-11 ug/ml range of human plasma adiponectin, 1 vial, lyophilized
- 8) Substrate I, 6 ml
- 9) Substrate II, 6 ml
- 10) Stop solution, 12 ml

Reagents Description

Antigen coated 96-well plate, 12X 8-well strips, with absorbed recombinant human adiponectin

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

5X Diluent, for sample and reagent dilution

1X antibody, polyclonal antibody against human adiponectin

100X detector, HRP conjugated anti-rabbit IgG

Substrate I and II, chromogenic reagents

Stop solution, 1M H₃PO₄

Standard, 1ug, recombinant human adiponectin

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 detector antibody and substrate.
Reagent reservoirs.
Microwell or microstrip plate reader 450 nm
Deionized water

Plasma Collection and Storage

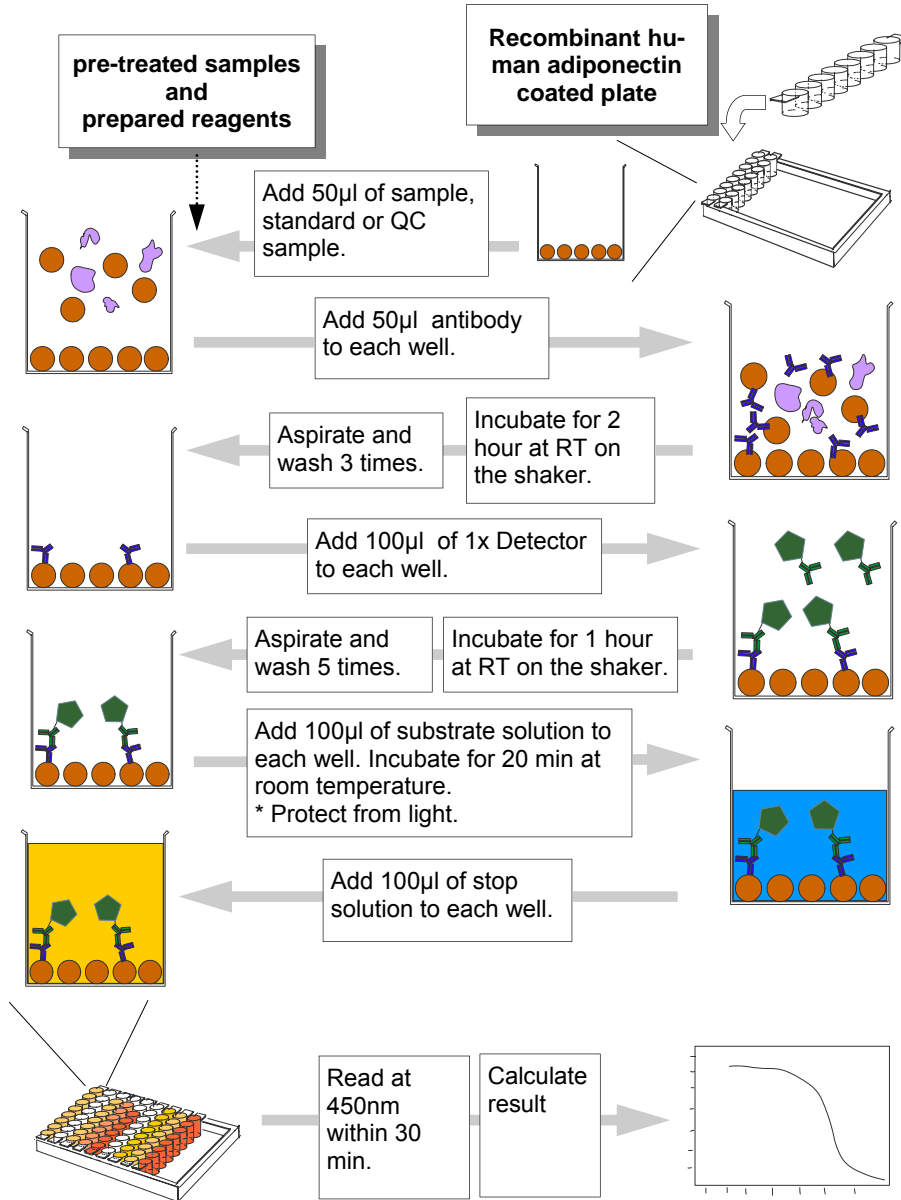
Blood samples for measurement of plasma adiponectin are collected in vacutainer tube and all tubes are centrifuged at 4°C for collection of plasma. These are stored at -80°C until analyses

Assay Procedure

1. Preparation of Reagents

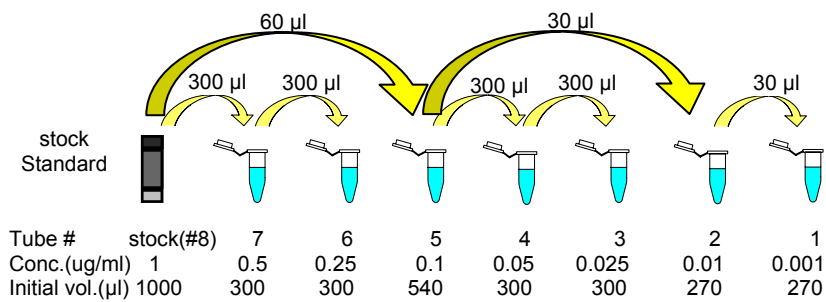
- 1) Allow all samples and kit components to equilibrate to room temperature (20-25°C).
- 2) Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use (See table on page 7). It is recommended that standards and samples be run in duplicate.
- 3) Prepare **1X 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.

Flow Chart of Assay Procedure



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 ₂ O	Stable for 1 month at RT
1X Diluent	2.5 ml	0.5 ml of 5X Diluent	2.0 ml of ddH ₂ O	in the case of 5 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

- 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 the Standard vial to make a stock standard concentration of 1ug/ml(#8). Mix well. A recommended dilution scheme is as follows :
- Label 7 microcentrifuge tubes #1-7. Add 1X Diluent to the microcentrifuge tubes #1-7 as shown on page 8, respectively.
 - Add 300 µl of the stock Standard solution(#8) to tube #7 and vortex. This is Standard tube #7 with a concentration of 0.5ug/ml. Standard #6 is then prepared by performing a 1:2 dilution of the preceding standard.
 - Standard #5 is prepared by performing a 1:10 dilution of the stock standard solution and vortex and Standards #4 to #3 are then prepared by performing a 1:2 dilution of the preceding standard.
 - Standard #2 is prepared by performing a 1:10 dilution of the preceding solution #5 and vortex and standard #1 is then prepared by performing a 1:10 dilution of the preceding solution #2 and vortex.
 - Use 50µl of the final diluted standards(#8 to #1) for ELISA.



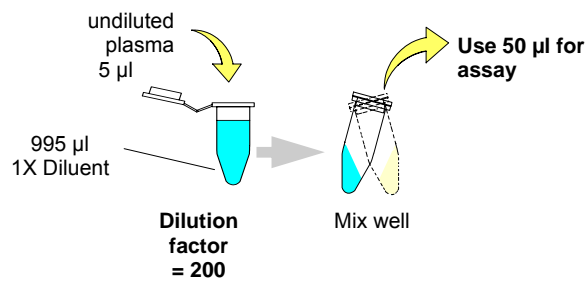
8) Reconstitute QC sample in 1 ml of deionized water.

2. Sample dilution

1) Dilute samples 1:200 with 1X Diluent (for example, 5 ul sample plus 995 ul 1X Diluent, final 1:200)

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

2) Use 50ul of the final diluted plasma for ELISA.



3. Experiment procedure

- 1) Remove the appropriate number of microwell strips from the sealed foil pouch.
- 2) Pipette 50 μ l of standards 1 to 8, the reconstituted QC sample and pre-treated plasma sample into the recombinant protein-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.
- 3) Add 50 μ l antibody to each well.
- 4) Incubate at RT for 2 hour on the shaker.
- 5) Remove the solution and wash 3 times with 250 μ l of 1X Wash Solution to each well.
- 6) Add 100 μ l 1X Detector to each well
- 7) Incubate at RT for 1 hour on the shaker.
- 8) Remove the solution and wash 5 times with 250 μ l of 1X Wash Solution to each well.

9) Add 100 μ l of the Substrate Solution to each well.

10) Incubate at room temperature for 20 min.

* Protect from light.

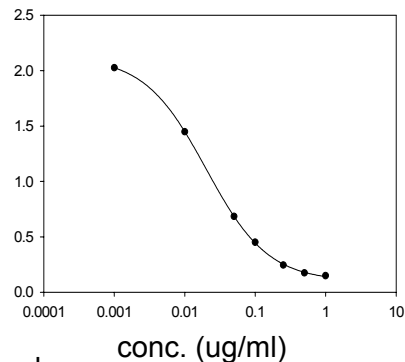
11) Using the multi-channel pipette, add 100 μ l Stop Solution to each well.

12) Read at 450 nm. OD

13) The dose-response curve of this assay fits best to a sigmoidal 4-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4-parameter logistic function.

14) A measurable range is typically shown between 0.001 μ g/ml and 1 μ g/ml.

The adiponectin concentrations calculated must be multiplied by dilution factor to obtain the concentrations of the undiluted samples (Dilution factor of lyophilized QC sample is 200).



Performance Characteristics

1) Sensitivity

The limit of detection: 100 pg/ml

2) Precision

a. Intra-Assay (precision within an assay)

4 samples were tested 5 times to assess intra-assay precision.

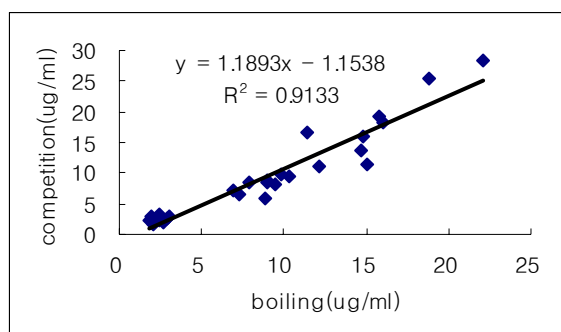
Sample	Mean (µg/ml)	SD (µg/ml)	CV(%)
1	2.96	0.14	4.69
2	6.68	0.22	3.36
3	11.94	0.58	4.84
4	22.50	0.69	3.08

b. Inter-Assay (precision between assay)

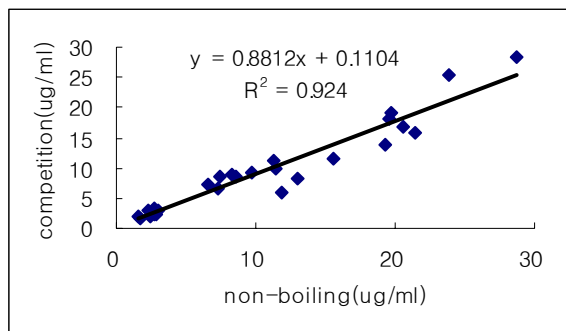
4 samples were tested 5 times to assess inter-assay precision.

Sample	Mean (µg/ml)	SD (µg/ml)	CV(%)
1	3.22	0.16	5.05
2	7.30	0.13	1.76
3	11.03	0.30	2.75
4	25.04	0.50	2.00

3) Correlation between boiling and competitive ELISA system



4) Correlation between non-boiling and competitive ELISA system



5) Comparison of normal group with diabetic group

Sample No.	conc. (ug/ml) of normal group	conc. (ug/ml) of diabetic group
1	12.941	1.035
2	10.722	1.322
3	9.612	0.984
4	13.018	2.523
5	19.463	2.384
6	12.375	1.999

6) specificity

- a. No cross reaction mouse and rat sera
- b. Cross Reactivity

Analyte	Max. Conc. (ug/ml)	Cross Reactivity (%)
Human Adiponectin	0.1	100
Mouse Adiponectin	0.1	N. R
Rat Adiponectin	0.1	N. R
Human Resistin	1	N. R
Human RELM α	1	N. R
Human RELM β	1	N. R
Human Leptin	1	N. R
Human TNF- α	1	N. R

7) Linearity

N. R : No Cross-reactivity

Effect of Serum Dilution

Sample No.	Serum Dilution	Expected (ug/ml)	Observed (ug/ml)	% Of Expected
1	1	12	12	100
	1/2	6	5	83
	1/4	3	2.4	80
	1/8	1.5	1.2	80
2	1	30.2	30.2	100
	1/2	15.1	14.8	98
	1/4	7.6	6.4	85
	1/8	3.8	3.6	95

$$\% \text{ of expected} = \text{observed} / \text{expected} \times 100\%$$

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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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