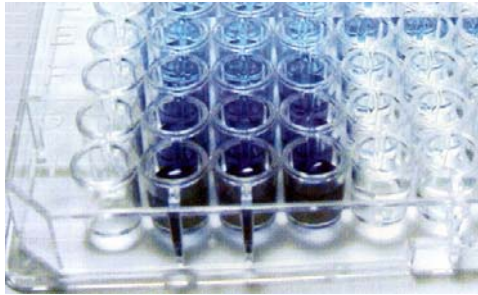


# Human Adiponectin ELISA Kit

Cat. No. A0512EK



*Instruction Manual*  
**Version 2.1.3**

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 an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of adiponectin in human serum, plasma or various tissue or cell culture supernatants. The human adiponectin ELISA system was originally described in the following paper entitled "plasma resistin concentrations measured by enzyme-linked immunosorbent assay using a newly developed monoclonal antibody are elevated in Individuals with type-2 diabetes mellitus" J. Cli. Endo. Meta. 89:150-156, 2004 and further modified.

## Kit Components

- 1) Antibody coated 96-well plate, 12X 8-well strips
- 2) 5X Wash concentrate, 100 ml
- 3) 5X Diluent, 50 ml
- 4) Secondary 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 µg/ml range of human plasma adiponectin, 1vial, lyophilized
- 8) Substrate I, 6 ml
- 9) Substrate II, 6 ml
- 10) Stop solution, 12 ml

## Reagents Description

**Antibody coated 96-well plates**, 12X 8-well strips, with absorbed monoclonal antibody against human adiponectin

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

**5X Diluent**, for sample and reagent dilution

**1X Secondary antibody**, polyclonal antibody against human adiponectin

**100X detector**, HRP conjugated anti-rabbit IgG

**Substrate I and II**, chromogenic reagents

**Stop solution**, 1M H<sub>3</sub>PO<sub>4</sub>

**Standard, 64.0 ng**, 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 temperature 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

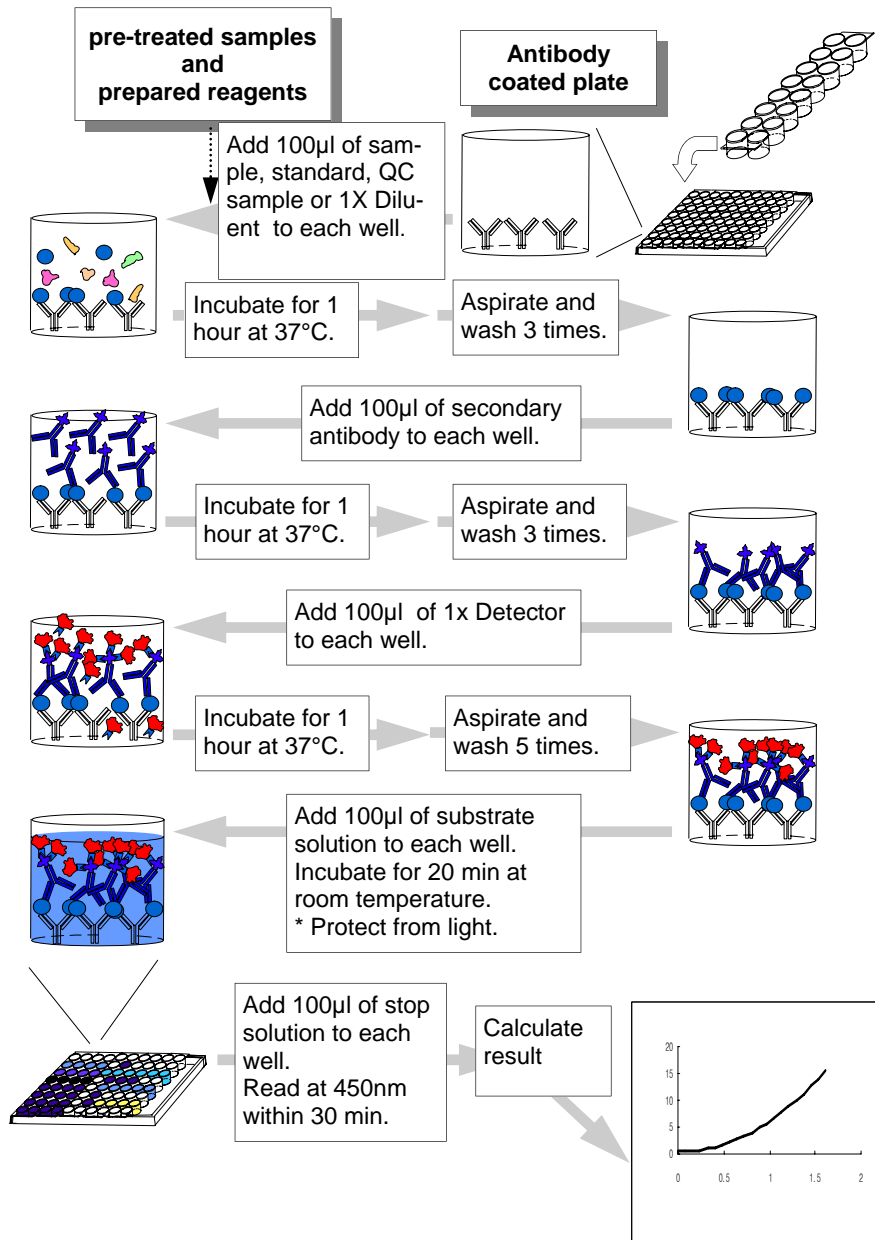
Blood samples for measurement of plasma adiponectin are collected in vacutainer tube and all tubes are centrifuged at 4°C for collection of plasma. These are stored at -80°C until analyses.

## 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

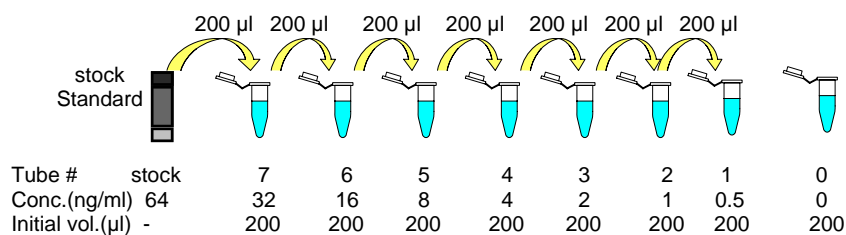


The amount of working reagents needed for 1 well				
Working reagents	Total volume needed	Stock solution added	Dilution solution added	Note
1X Wash Solution	2.8 ml	0.56 ml of 5X Wash Concentrate	2.24 ml of ddH <sub>2</sub> O	Stable for 1 month at RT
1X Diluent	2.5 ml	0.5 ml of 5X Diluent	2.0 ml of ddH <sub>2</sub> O	in the case of 10 µl sample
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 to the Standard vial to make a stock concentration of 64 ng/ml. Mix well. A recommended dilution scheme is as follows :

- 1) Label 8 microcentrifuge tubes #0-7. Add 200 µl of the 1X Diluent to the microcentrifuge tubes #0-7.
- 2) Add 200 µl of the stock Standard solution to tube #7 and vortex. This is Standard tube #7 with a concentration of 32 ng/ml.
- 3) 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.



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

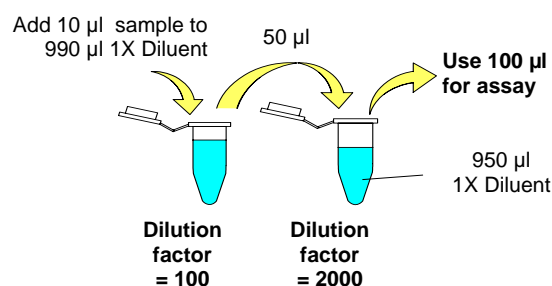
## 2) Sample dilution

Step 1. Dilute samples 1:100 with 1X Diluent (for example, 10  $\mu$ l sample plus 990  $\mu$ l 1X Diluent, final 1:100)

Step 2. Dilute the samples (from step 1) 1:20 with 1X Diluent (for example, 50  $\mu$ l step 1 sample plus 950  $\mu$ l 1X Diluent, final 1:2000)

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

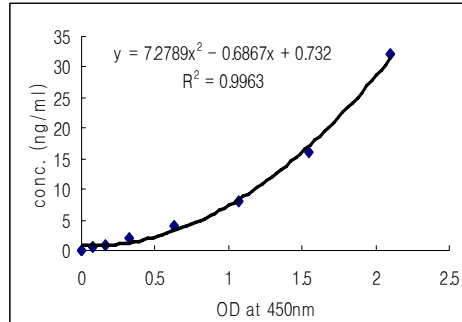
Step 3. Use 100 $\mu$ l of the final diluted sample for ELISA.



## 3) Experiment procedure

1. Remove the appropriate number of microwell strips from the sealed foil pouch.
2. Pipette 100  $\mu$ l of Standards #0 to #7, the reconstituted QC sample and pre-treated 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  $\mu$ l of 1X Wash Solution per well.
5. Add 100  $\mu$ l Secondary Antibody to each well.
6. Incubate at 37°C for 1 hour.
7. Remove the solution and wash 3 times with 250  $\mu$ l of 1X Wash Solution per well.
8. Add 100 $\mu$ l 1X Detector to each well.
9. Incubate at 37°C for 1 hour.
10. Remove the solution and wash 5 times with 250  $\mu$ l of 1X Wash Solution per well.

11. Using the multi-channel pipette, 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 a Standard curve by plotting the known concentrations (Y) of Standard versus the absorbances (X) of Standard. A measurable range is typically shown between 0.5 ng/ml and 32 ng/ml.
17. Calculate the adiponectin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
18. 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 2000).



## 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	1.86	0.07	3.82
2	5.90	0.23	3.84
3	8.50	0.28	3.31
4	23.36	0.69	2.97

**b. Inter-Assay (precision between assays)**

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

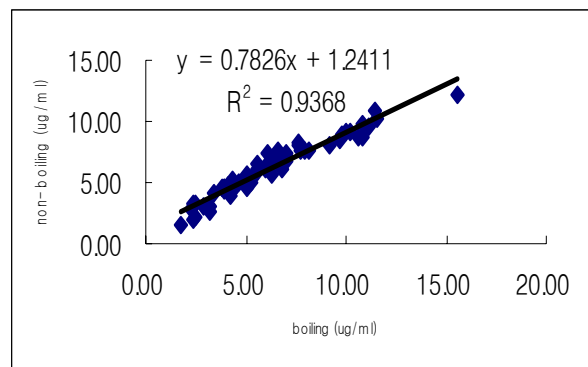
Sample	Mean (µg/ml)	SD (µg/ml)	CV(%)
1	2.50	0.13	5.15
2	7.78	0.43	5.50
3	11.10	0.44	3.97
4	24.82	0.70	2.84

**3) Recovery**

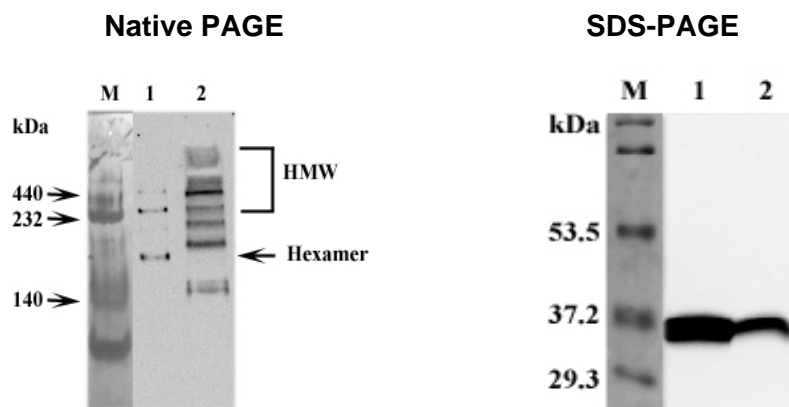
The recovery of Adiponectin spiked to four different levels in five different plasma samples throughout the range of assay was evaluated.

Sample No.	Average recovery (%)	Range (%)
1	99.6	96~105
2	99.8	96~104
3	100.2	97~102
4	92.5	88~95
5	91.8	86~100

**4) Correlation between boiling and non-boiling ELISA system**



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



1. FLAG-tagged human adiponectin
2. Human serum

**6) Specificity**

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

Analyte	Max. Conc. (ng/ml)	Cross Reactivity (%)
Human Adiponectin	10	100
Mouse Adiponectin	10	N. R.
Rat Adiponectin	10	N. R.
Human Resistin	100	N. R.
Human RELM-β	100	N. R.
Human Leptin	100	N. R.
Human TNF-α	100	N. R.
IL-6	100	N. R.

N. R.: No Cross-reactivity

## References

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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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