



Human Adiponectin ELISA Kit

Cat. No. CYT350

**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.

Test Principle

This kit is enzyme-linked immunosorbent assay (ELISA) for quantitative determination of Adiponectin in human serum or plasma. Monoclonal antibody specific for human Adiponectin has been precoated 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 biotinylated anti-human adiponectin polyclonal antibody. HRP conjugated streptavidin is added. After washing, a substrate solution is added. The colors develop in proportion to the bound adiponectin quantity. The color development is stopped and the intensity of color is measured.

Application

The CHEMICON® Human Adiponectin ELISA Kit is designed for *in vitro* quantitative detection of adiponectin in serum or plasma samples of human origin. There are enough reagents included in this kit for one 96-well immunoassay plate. Running duplicate wells for samples and standards is recommended.

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Analytical Sensitivity and Detection Limits

| | |
|------------------------|--|
| Sensitivity: | 100 pg/mL |
| Intra-assay Variation: | $\pm 3.84\%$ (5.90 $\mu\text{g/mL}$) |
| Inter-assay Variation: | $\pm 5.50\%$ (7.78 $\mu\text{g/mL}$) |
| Recovery: | 88-105% for spiked samples |
| Cross-reactivity: | No reactivity detected with 10 ng/mL of mouse or rat adiponectin, or 100 ng/mL of human Resistin, RELM- β , Leptin, IL-6, or TNF- α . |

Kit Components

1. 96-Well Plate: (Catalog No. CYT350a) 12x8 well strips coated with absorbed monoclonal antibody against human adiponectin.
2. Wash Concentrate: (Catalog No. CYT350b) One 100 mL (5X) bottle.
3. Assay Diluent: (Catalog No. CYT350c) One 50 mL (5X) bottle.
4. Secondary Antibody: (Catalog No. CYT350d) Anti-human Adiponectin biotinylated Polyclonal Antibody, 12 mL.
5. QC Sample: (Catalog No. CYT350e) Positive control, human serum (see vial label for value).
6. Detector: (Catalog No. CYT350f) One 150 μL (100X) bottle of HRP conjugated Streptavidin.
7. Adiponectin Standard (Recombinant Human): (Catalog No. CYT350g) One 64.0 ng vial (Lyophilized).
8. Substrate I: (Catalog No. CYT350h) One 6 mL bottle.
9. Substrate II: (Catalog No. CYT350i) One 6 mL bottle.
10. Stop Solution: (Catalog No. CYT350j) One 12 mL bottle.

Materials Not Supplied

1. Precision single and multi-channel pipettes.
2. Disposable pipette tips.
3. Microtubes or equivalent for preparing dilutions.
4. Disposable plastic containers for preparing working detector antibody and substrate.
5. Reagent reservoirs.
6. Microwell or microstrip plate reader 450 nm.
7. Deionized water.

Precautions

- *Human Source Material:* The human source material supplied has been tested by an FDA approved method and found to be non-reactive for HIV-1/2 Antibody, HCV Antibody, a Serologic Test for Syphilis (STS), and Hepatitis B Surface Antigen (HBsAg). However, all materials should be handled carefully in accordance with good laboratory practices.
- The instructions provided have been designed to optimize the kit's performance. Deviation from the instructions may result in suboptimal performance of the kit and the failure to produce accurate data.

Preparation of Reagents

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

Dilute 5X Diluent 1:5 with deionized water (1 part 5X Diluent with 4 parts deionized water).

5. **Detector (1X)**

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. **Substrate Solution**

Freshly prepare just before use the by adding one part Substrate I to one part Substrate II.

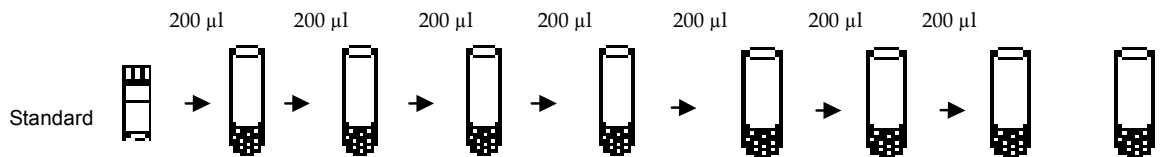
7. **Adiponectin Standard**

Prepare working aliquots of the Standard as follows:

Briefly centrifuge the Standard vial. 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:

- a. Label 8 microcentrifuge tubes #0-7. Add 200 μ L of the 1X Diluent to the microcentrifuge tubes # 0-7.
- b. Add 200 μ L of the stock Standard solution to tube # 7 and vortex. This is Standard tube # 7 with a concentration of 32 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.



Initial Volume (μ L):

1000 200 200 200 200 200 200 200

Concentration (ng/mL):

64 32 16 8 4 2 1 0.5 0.0

Standard Number:

#7 #6 #5 #4 #3 #2 #1 0

Figure 1: Serial Dilution of Adiponectin Standard

8. QC Sample

Reconstitute QC sample in 1 mL of deionized water.

Serum Collection and Storage

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

Storage

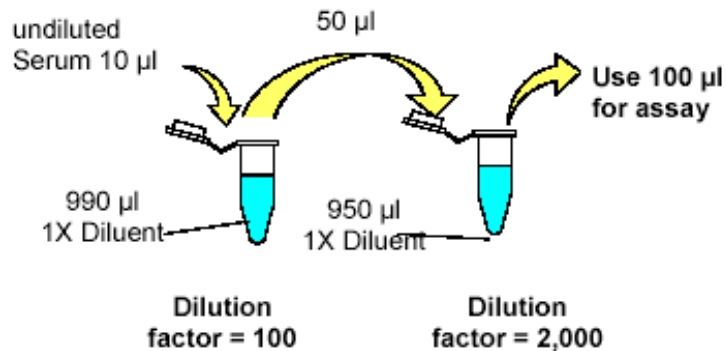
Reagents must be stored at 2° to 8°C when not in use. Reagents must be brought to room temperature before use. Do not expose reagents to temperatures greater than 25°C. Diluted wash solution may be stored at room temperature for up to one month.

Preparation of Samples

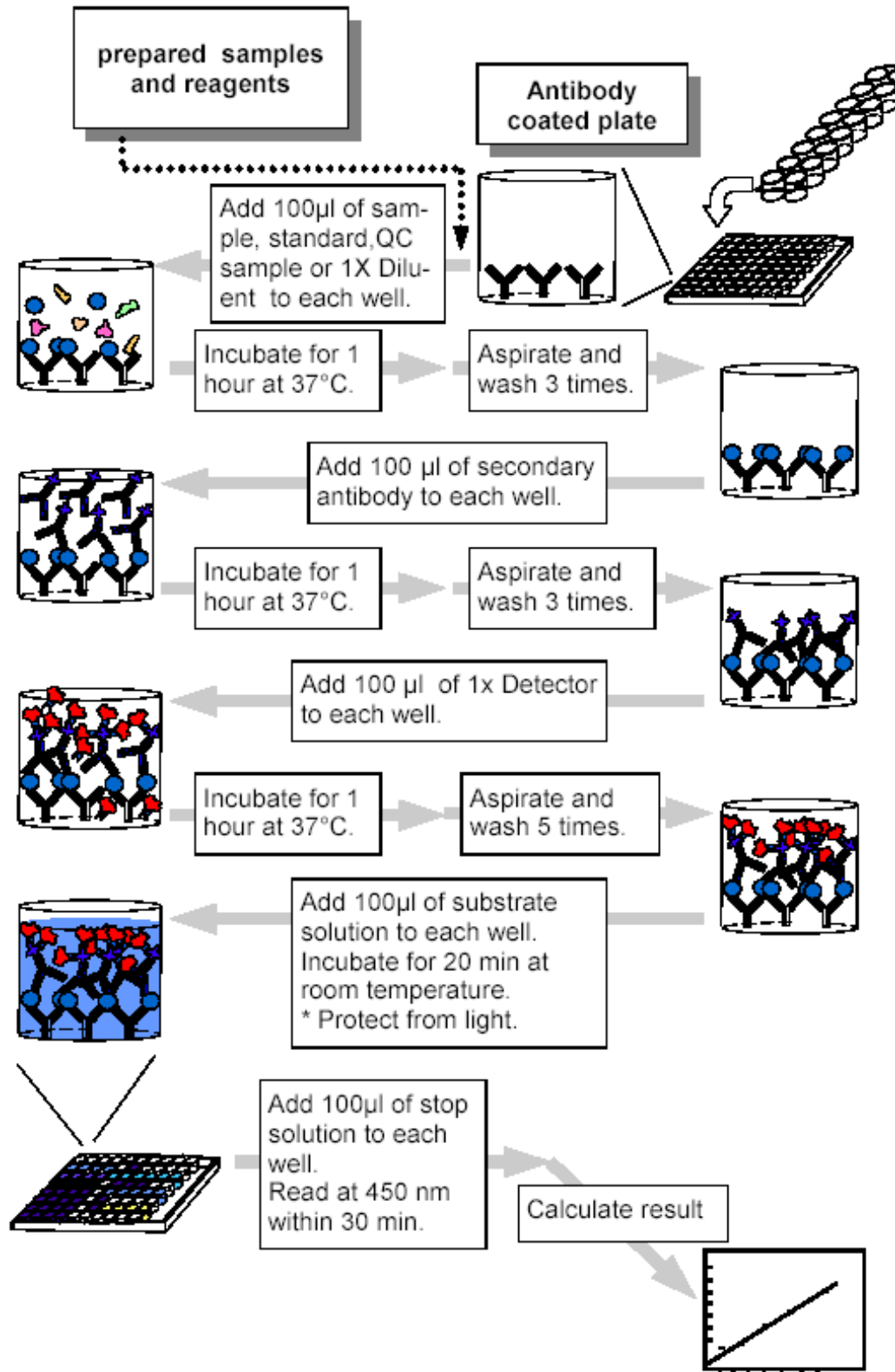
1. Dilute sample 1:100 with 1X Diluent (example, 10 µL sample plus 990 µL 1X Diluent; dilution factor=100) and mix well.
2. Dilute the sample from step 1 1:20 with 1X Diluent (example, 50 µL of the step 1 sample plus 950 µL 1X Diluent, dilution factor=2,000).

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

NOTE: Do not dilute the QC Sample included in the kit.



Flow Chart of Assay Procedure

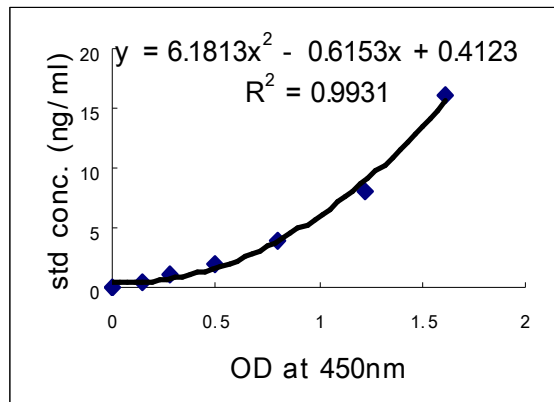


Assay Instructions

1. Remove the appropriate number of microwell strips from the sealed foil pouch.
2. Pipette 100 μL of standard, 0 through 7, the reconstituted QC sample and diluted serum or 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 100 μL 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. ***NOTE: Protect from light.**
13. Using the multi-channel pipette, add 100 μL Stop Solution to each well.
14. Read at 450 nm.

Calculation of Results

1. Subtract the absorbance of the blank from the readings for each standard and sample.
2. 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 0.5 ng/ml and 32 ng/ml.
3. Calculate the adiponectin concentrations of samples by interpolation of the regression curve formula as shown below in the form of a quadratic equation.
4. The adiponectin concentrations calculated for the unknown samples and QC sample must be multiplied by the dilution factor [see **Preparation of Samples**] to obtain the concentrations of the undiluted samples.



Sample standard curve.

References

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2. Hu E, Liang P, Spiegelman BM (1996). AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem* **18**:10697-10703.
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4. Byung-Soo Youn, Kang-Yeol Yu, Hong Je Park, Nam Seok Lee, Sung-Shik Min, Moon Yoen Youn, Young Min Cho, Young Joo Park, Seong Yeon Kim, Hong Kyu Lee, Kyung Soo Park. (2004). Plasma resistin concentrations are elevated in Individuals with Type-2 diabetes mellitus. *J Cli Endo Meta* **89**:150-156.
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|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----------|-----------|-----------|--|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| A | | | | | | | | | | | | | |
| B | | | | | | | | | | | | | |
| C | | | | | | | | | | | | | |
| D | | | | | | | | | | | | | |
| E | | | | | | | | | | | | | |
| F | | | | | | | | | | | | | |
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|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----------|-----------|-----------|--|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| A | | | | | | | | | | | | | |
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| H | | | | | | | | | | | | | |

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. |

Warranty

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