

# **Rapid Human Adiponectin ELISA Kit**

(Catalog Number: 31012)

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

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## **INTRODUCTION**

Adiponectin, also known as apM1, Acrp30, GBP28 and adipoQ, is a circulating hormone predominantly produced from adipose tissue<sup>1</sup>. 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<sup>2</sup>, improve insulin sensitivity<sup>3</sup>, alleviate fatty liver<sup>4</sup> and prevent atherosclerosis<sup>5</sup>. The protein is post-translationally modified by hydroxylation and glycosylation<sup>6</sup>, and forms three different oligomeric complexes in the circulation<sup>7</sup>. Many clinical studies demonstrated that plasma adiponectin is a useful biomarker for metabolic syndrome, nonalcoholic steatohepatitis and certain type of cancers<sup>1</sup>. 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<sup>8</sup>. 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) designed for the quantitative detection of human adiponectin in samples in 1 hour. A mouse monoclonal antibody specific to human adiponectin has been pre-coated onto a microtiter plate. The user pipettes standards and samples into the wells and any human adiponectin present is sandwiched by the immobilized antibody and a second horseradish peroxidase (HRP)-linked monoclonal antibody specific to human adiponectin that is co-incubated with the samples. After wash step to remove any unbound reagents, an HRP substrate solution is added and color develops in proportion to the amount of human 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 human adiponectin, the unknown sample concentration can be interpolated from a reference curve included in each assay.

## **INTENDED USE**

This Rapid Human Adiponectin ELISA kit is designed for quantification of human adiponectin in serum, plasma, and adipocyte extracts or cell culture media samples.

## **REAGENTS SUPPLIED**

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

1. Microtiter Strips (96 wells), coated with a mouse monoclonal antibody against human adiponectin, sealed
2. 10×Wash buffer, 20 mL
3. 5×Assay buffer, 30 mL

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4. 100×Detection antibody solution, a mouse monoclonal antibody against human adiponectin conjugated with horseradish peroxidase, 0.25 mL
5. Human adiponectin standard, 100 ng of recombinant human adiponectin, lyophilized
6. Substrate solution, 12 mL, ready for use
7. Stop solution, 12 mL, ready for use
8. Plate cover

### **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
7. Horizontal micro-plate shaker capable of 600 rpm.

### **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 human adiponectin microplate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°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 (30 mL) with 120 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. 1×Assay buffer may be stored at 2-8°C for up to one month.

#### **B. 1×Wash buffer**

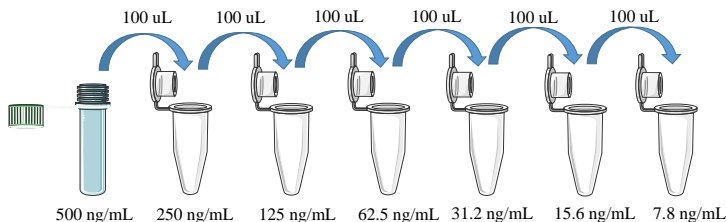
Prepare 1×Wash buffer by mixing the 10×Wash buffer (20 mL) with 180 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. 1×Wash buffer may be stored at 2-8°C for up to one month.

#### **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, 200 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 to 2-8°C immediately after the necessary volume is removed.

## PREPARATION OF STANDARDS AND SAMPLES

**Human Adiponectin Standards:** Reconstitute the lyophilized standard with 200  $\mu\text{L}$  of 1 $\times$ Assay buffer to generate a standard stock solution of 500 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 100  $\mu\text{L}$  of 1 $\times$ Assay buffer to 250, 125, 62.5, 31.2, 15.6, 7.8 ng/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1 $\times$ Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and stored at  $-20^{\circ}\text{C}$  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 **100-fold** dilution in this assay. A suggested dilution step is to add 10  $\mu\text{L}$  of sample to 990  $\mu\text{L}$  of 1 $\times$ Assay buffer. Cellular extract and culture media dilutions will vary and need to be optimized by the user, also use 1 $\times$ 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 10  $\mu\text{L}$  of standard or sample to its respective well.
2. Add 200  $\mu\text{L}$  of the 1 $\times$ Detection antibody solution to each well, seal the plate with a plate cover. Incubate at room temperature for 30 minutes, shaking the plate at 600 rpm on a horizontal micro-plate shaker.
3. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300  $\mu\text{L}$  of 1 $\times$ Wash buffer to each well and incubate for 30 seconds. Discard the 1 $\times$ 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.
4. Add 100  $\mu\text{L}$  of Substrate solution to each well. Incubate for 15 minutes at room temperature. **Protect from light.**
5. Add 100  $\mu\text{L}$  of Stop solution to each well. Gently tap the plate frame for a few seconds to ensure thorough mixing.
6. Measure absorbance of each well at 450 nm immediately.

## CALCULATION

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

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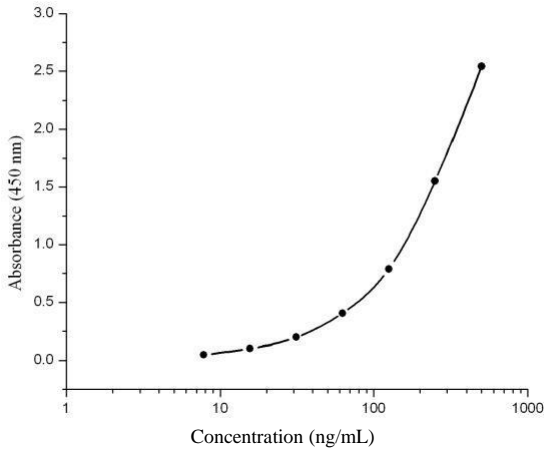
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against adiponectin concentrations (x-axis). The best fitting line can be generated with any curve-fitting software, any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine adiponectin concentration of samples from the 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	0.056	0
7.8	0.105	0.049
15.6	0.158	0.102
31.2	0.255	0.199
62.5	0.463	0.407
125	0.845	0.789
250	1.608	1.552
500	2.599	2.543

Human adiponectin standard curve (4-parameter)



## ASSAY CHARACTERISTICS

### A. Sensitivity

The lowest level of adiponectin that can be detected by this assay is 7.8 ng/mL.

### B. Specificity

The antibody pair used in this assay is specific to human adiponectin and does not cross-react with mouse and rat adiponectin, and other cytokine or hormone molecules tested, including human resistin, TNF $\alpha$ , ANGPTL4, insulin, leptin and IL6.

### C. Precision

Intra-assay Precision (Precision within an assay) C.V. <10%.

Inter-assay Precision (Precision between assays) C.V. <10%.

### D. Recovery

The recovery of the assay was determined by adding various amounts of adiponectin to a sample. The measured concentration of the spiked sample in the assay was compared to the expected concentration. The average recovery was 91%.

## REFERENCES

1. Trujillo ME, Scherer PE. (2005) *J Intern Med.* 257:167-175.
2. Berg AH. et al. (2001) *Nat Med.* 7:947-953.
3. Yamauchi T. et al. (2001) *Nat Med.* 7:941-946.
4. Xu A. et al. (2003) *J Clin Invest.* 112:91-100.
5. Okamoto Y. et al. (2002) *Circulation.* 106:2767-2770.
6. Wang Y. et al. (2002) *J Biol Chem.* 277:19521-19529.
7. Xu A. et al. (2005) *J Biol Chem.* 280:18073-18080.
8. Maeda N. et al. (2001) *Diabetes.* 50:2094-2099.

### SUMMARY OF ASSAY PROCEDURE

Add 10  $\mu$ L of standard or sample to each well.



Add 100  $\mu$ L of 1 $\times$ Detection antibody solution to each well.



Incubate at room temperature for 30 minutes (600 rpm).



Aspirate and wash each well 3 times.



Add 100  $\mu$ L of Substrate solution to each well.



Incubate at room temperature for 15 minutes.



Add 100  $\mu$ L of Stop solution to each well.



Measure absorbance of each well at 450 nm.



Calculation