# Human Adiponectin Immunoassay Kit

Catalogue Number: 31010

For the quantitative determination of human adiponectin concentrations
in serum, plasma, adipocyte extract and cell culture supernate samples.

This package insert must be read in its entirety before using this product
Use only the current version of product data sheet enclosed with the kit

Version: 3.0

**FOR RESEARCH USE ONLY**
**NOT FOR USE IN DIAGNOSTIC PROCEDURES**

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## INTRODUCTION

Adiponectin, also known as apM1, Acrp30, GBP28 and adipoQ, is a circulating hormone predominantly produced from adipose tissue[1]. Many pharmacological studies demonstrated that this protein possesses potent anti-diabetic, anti-atherogenic and anti-inflammatory functions. Supplement of adiponectin protein can decrease blood glucose[2], improve insulin sensitivity[3], alleviate fatty liver[4] and prevent atherosclerosis[5]. The protein is post-translationally modified by hydroxylation and glycosylation[6], and forms three different oligomeric complexes in the circulation[7].

Many clinical studies demonstrated that plasma adiponectin is an useful biomarker for metabolic syndrome, nonalcoholic steatohepatitis and certain type of cancers[1]. Decreased circulating levels of plasma adiponectin (hypoadiponectinaemia) are associated with increased body mass index (BMI), decreased insulin sensitivity, less favourable 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[8]. 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 quantitative sandwich ELISA using monoclonal antibodies against human adiponectin. The immunoplate is pre-coated with a monoclonal antibody specific for human adiponectin. Standards and samples are pipetted into the wells and any human adiponectin present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked monoclonal antibody specific for human adiponectin is added to the wells. After a final wash step, an HRP substrate solution is added and colour develops in proportion to the amount of human adiponectin bound initially. The assay is stopped and the optical density of the wells 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 Human Adiponectin ELISA kit is designed for quantification of human adiponectin in serum, plasma, adipocyte extract and cell culture media samples.

## REAGENTS SUPPLIED

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

1. Micro-titre strips (96 wells)-Coated with a mouse monoclonal antibody against human adiponectin, sealed.
2. 10×Wash buffer-30 ml.
3. 5×Assay buffer-30 ml.
4. 100×Detection antibody solution-A mouse monoclonal antibody against human adiponectin conjugated with horseradish peroxidase, 0.12 ml.
5. Human adiponectin standard-100 ng of recombinant human adiponectin in a buffered protein base, lyophilised.
6. Substrate solution- 12 ml, ready for use.
7. Stop solution-12 ml, ready for use.

## 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.

## 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, 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. The 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 (30 ml) with 270 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. The 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, 100 l 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 solution to 2-8°C immediately after the necessary volume is removed.

## PREPARATION OF STANDRADS AND SAMPLES

**Human Adiponectin Standards:** Reconstitute the lyophilised standard with 1 ml of 1×Assay buffer to generate a standard stock solution of 100 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare serially diluted standards using 1×Assay buffer as follows:

| Standard volume | Volume of 1×Assay buffer | Concentration |
|---|---|---|
| 100.0 ng/ml stock | - | 100.0 ng/ml |
| 250 1 of 100.0 ng/ml | 250 1 | 50.0 ng/ml |
| 250 1 of 50.0 ng/ml std. | 250 1 | 25.0 ng/ml |
| 250 1 of 25.0 ng/ml std. | 250 1 | 12.5 ng/ml |
| 250 1 of 12.5 ng/ml std. | 250 1 | 6.25 ng/ml |
| 250 1 of 6.25 ng/ml std. | 250 1 | 3.12 ng/ml |
| 250 1 of 3.12 ng/ml std. | 250 1 | 1.56 ng/ml |

1×Assay buffer serves as the zero standard (0 ng/ml). The reconstituted standard stock should be aliquoted and frozen at -20°C for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

### Sample preparation

Serum or plasma sample is generally required a 1000-fold dilution in this assay. A two-step dilution is suggested. Step1- Add 10 μl of sample to 490 μl of 1× Assay buffer to give a 50-fold diluted sample solution. Step 2- Add 20 μl of the 50-fold diluted sample solution to 380 μl of 1×Assay buffer to give a final 1000-fold diluted sample solution. Cellular extract and culture media dilutions will vary and need to be optimized by the user, also use 1×Assay buffer to prepare these samples.

## ASSAY PROCEDURE

*It is recommended that all standards and samples be assayed in duplicate.*

1. Add 100 μl of standard or sample per well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 μl of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×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.
3. Add 100 μl of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 3 times as in step 2.
5. Add 100 μl of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
6. Add 100 μl of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
7. Measure absorbance of each well at 450 nm immediately.

## CALCULATION

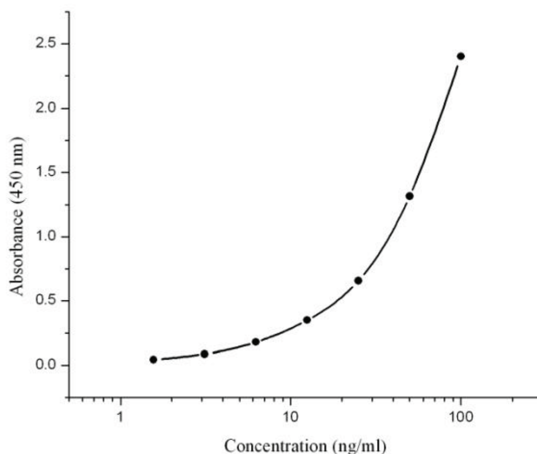
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human adiponectin concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine human adiponectin concentration of samples from 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 | 0.068 | 0 |
| 1.56 | 0.112 | 0.044 |
| 3.12 | 0.156 | 0.088 |
| 6.25 | 0.248 | 0.18 |
| 12.5 | 0.418 | 0.35 |
| 25 | 0.724 | 0.656 |
| 50 | 1.384 | 1.316 |
| 100 | 2.469 | 2.401 |

Human adiponectin standard curve (4-parameter)



## ASSAY CHARACTERISTICS

**A. Sensitivity:** The lowest level of human adiponectin that can be detected by this assay is 1.56 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 including human resistin, TNF , ANGPTL4, insulin, leptin and IL6.

**C. Precision:** The assay variations of this ELISA kits were studied on four human serum samples with varying concentrations of endogenous adiponectin. The mean within variation was calculated from results of five duplicate determinations in each assay of the indicated samples. The mean between variations of each sample was calculated from results of four separate assays with duplicate samples in each assay.

| Sample No. | Mean Adiponectin Levels ( g/ml) | Within% CV | Between% CV |
|---|---|---|---|
| 1 | 9.21 | 4.02 | 4.97 |
| 2 | 21.33 | 3.65 | 4.68 |
| 3 | 5.32 | 3.27 | 4.53 |
| 4 | 15.72 | 4.18 | 5.01 |

**D. Recovery:** Varying amounts of human adiponectin were added to three human serum samples and the Adiponectin content was determined in three separate assays. The % of recovery = observed adiponectin concentrations/expected adiponectin concentrations x 100%.

| | Average recovery | Range (%) |
|---|---|---|
| 1 | 99.3 | 97-104 |
| 2 | 99.8 | 98-103 |
| 3 | 101.1 | 99-105 |
| 4 | 100.2 | 98-104 |

**REFERENCES**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## SUMMARY OF ASSAY PROCEDURE

Add 100 µl of Standard or sample per well.

⊠

Incubate at room temperature for 1 hour.

⊠

Aspirate and wash each well three times.

⊠

Add 100 µl of 1 ×Detection antibody solution to each well.

⊠

Incubate at room temperature for 1 hour.

⊠

Aspirate and wash each well three times.

⊠

Add 100 µl of Substrate solution to each well.

⊠

Incubate at room temperature for 15 minutes.

⊠

Add 100 µl of Stop solution to each well.

⊠

Measure absorbance of each well at 450 nm.

⊠

Calculation