Rapid Human Adiponectin Immunoassay kit

Catalogue Number: 31012

For the quantitative determination of human adiponectin concentrations in serum, plasma 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 posttranslationally 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 sandwich 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 micro-titre plate. The user pipettes standards and samples into the wells and any human adiponectin present is sandwiched by the immobilised 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 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 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. Micro-titre 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.
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 in a buffered protein base, lyophilised.
6. Substrate solution-12 ml, ready for use.
7. Stop solution-12 ml, ready for use.
8. Plate cover- 1.

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 deionised 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 STANDRADS AND SAMPLES

Human adiponectin standards: Reconstitute the lyophilised standard with 200 μl of 1×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. Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1×Assay buffer	Concentration
500 ng/ml stock	-	500 ng/ml
100 μl of 500.0 ng/ml stock	100 μl	250 ng/ml
100 μl of 250 ng/ml std.	100 μl	125 ng/ml
100 μl of 125 ng/ml std.	100 μl	62.5 ng/ml
100 μl of 62.5 ng/ml std.	100 μl	31.2 ng/ml
100 μl of 31.2 ng/ml std.	100 μl	15.6 ng/ml
100 μl of 15.6 ng/ml std.	100 μl	7.8 ng/ml

1×Assay buffer serves as the zero standard (0 ng/ml). The reconstituted standard stock should be aliquoted and frozen at -20ºC 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 100-fold dilution in this assay. A suggested dilution step is to add 10 µl of sample to 990 µl of 1×Assay buffer. 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 10 μl of standard or sample to its respective well.
2. Add 200 μl of the 1×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 μl of 1×Wash buffer to each well and incubate for 30 seconds. 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.
4. Add 100 μl of Substrate solution to each well. Incubate for 15 minutes at room temperature. **Protect from light.**
5. Add 100 μl 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.
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)
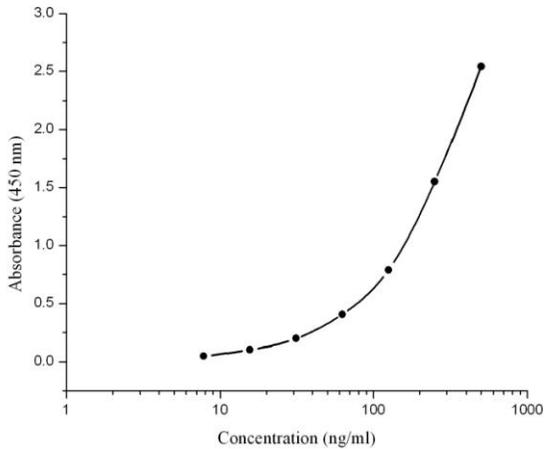
ASSAY CHARACTERISTICS

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, TNFα, 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 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
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5. Okamoto Y. et al. (2002) *Circulation.* 106:2767-2770.
6. Wang Y. et al. (2002) *J Biol Chem.* 277:19521-19529.
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8. Maeda N. et al. (2001) *Diabetes.* 50:2094-2099.

SUMMARY OF ASSAY PROCEDURE

Add 10 µl of Standard or sample per well.
↓
Add 200 µl of 1×Detection antibody to each well.
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Incubate at room temperature for 30 minutes (600 rpm).
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Aspirate and wash each well 3 times.
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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.
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Measure absorbance of each well at 450 nm.
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Calculation