Human Adiponectin   ELISA

Catalog Number	**Size**
EA100046	96 Tests

_____

Human Adiponectin   ELISA   KIT

For the quantitative determination of human Adiponectin concentrations in cell culture supernates, serum, and plasma.This package insert must be read in its entirety before using this product. If you have questions or experience problems with this product, please contact our Technical Support staff. Our scientists commit themselves to providing rapid and effective help.

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

INTRODUCTION

Adiponectin, alternately named Adipocyte complement-related protein of 30 kDa (Acrp30), adipoQ, adipose most abundant gene transcript 1 (apM1), and gelatin-binding protein of 28 kDa (GBP28), is an adipocyte-specific, secreted protein with potential roles in glucose and lipid homeostasis. Circulating Adiponectin levels are high, accounting for approximately 0.01% of total plasma protein (1-4). Adiponectin contains a modular structure that includes an N-terminal collagen-like domain followed by a C-terminal globular domain with significant sequence and structural resemblance to the complement factor C1q (1, 5, 6). Although they share little sequence identity, similar three-dimensional structure and certain conserved amino acid residues suggest an evolutionary link between the C1q-like domain of Adiponectin and members of the TNF superfamily (7). Adiponectin assembles into different complexes including trimers (low molecular weight), hexamers (middle molecular weight), and higher order oligomeric structures (high molecular weight) that may affect biological activity (1, 7, 8). Adiponectin is induced during adipocyte differentiation and its secretion is stimulated by insulin (1, 9). Two receptors for Adiponectin, termed AdipoR1 and AdipoR2, have been cloned (10). Although functionally distinct from G-protein-coupled receptors, the genes encode predicted proteins containing 7 transmembrane domains. AdipoR1 is highly expressed in skeletal muscle, while AdipoR2 is primarily found in hepatic tissues.

Injection of Adiponectin into non-obese diabetic mice leads to an insulin-independent decrease in glucose levels (11). This is likely due to insulin-sensitizing effects involving Adiponectin regulation of triglyceride metabolism (11). A truncated form of Adiponectin (gAdiponectin) containing only the C-terminal globular domain has been identified in the blood, and recombinant gAdiponectin has been shown to regulate weight reduction as well as free fatty acid oxidation in mouse muscle and liver (2, 12). The full-length recombinant Adiponectin protein is apparently less potent at mediating these effects (2, 12). The mechanism underlying the role of Adiponectin in lipid oxidation may involve the regulation of expression or activity of proteins associated with triglyceride metabolism including CD36, acyl CoA oxidase, AMPK, and PPARγ (12-14).

Although Adiponectin-regulation of glucose and lipid metabolism in humans is less clear, similar mechanisms may also be in place (15). A negative correlation between obesity and circulating Adiponectin has been well established (6, 16, 17), and Adiponectin levels increase concomitantly with weight loss (18). Decreased Adiponectin levels are associated with insulin resistance and hyperinsulinemia, and patients with type-2 diabetes are reported to exhibit decreased circulating Adiponectin (19, 20).

Thiazolidinediones, a class of insulin-sensitizing, anti-diabetic drugs, elevate Adiponectin in insulin-resistant patients (21). In addition, high Adiponectin levels are associated with a reduced risk of type-2 diabetes (22). Using magnetic resonance spectroscopy it has been demonstrated that intracellular lipid content in human muscle negatively correlates with Adiponectin levels, potentially due to Adiponectin-induced fatty acid oxidation (15).

Adiponectin may also play anti-atherogenic and anti-inflammatory roles. Adiponectin plasma levels are decreased in patients with coronary artery disease (20). Furthermore, neointimal thickening of damaged arteries is exacerbated in Adiponectin-deficient mice and is inhibited by exogenous Adiponectin (23). Adiponectin inhibits endothelial cell expression of adhesion molecules in vitro, suppressing the attachment of monocytes (24). In addition, Adiponectin negatively regulates myelomonocytic progenitor cell growth and TNF-α production in macrophages (25, 26).

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Adiponectin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Adiponectin present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for Adiponectin is added to the wells and binds to the combination of capture antibody- Adiponectin in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of Adiponectin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven Adiponectin standard dilutions and Adiponectin sample concentration determined.
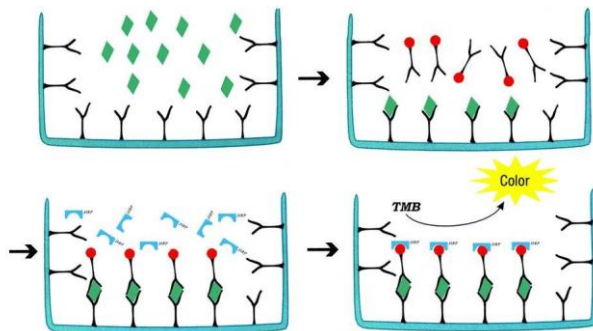
Figure 1:Schematic diagram of the assay

REAGENTS

1. Aluminium pouches with a Microwell Plate coated with antibody to human Adiponectin (8X12)

2. 2 vials human Adiponectin Standard lyophilized, 1000 pg/ml upon reconstitution

3. 2 vials concentrated Biotin-Conjugate anti-human Adiponectin antibody

4. 2 vials Streptavidin-HRP solution

5. 4 bottle Standard /sample Diluent

6. 1 bottle Biotin-Conjugate antibody Diluent

7. 1 bottle Streptavidin-HRP Diluent

8. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)

9. 1 vial Substrate Solution

10. 1 vial Stop Solution

11. 4 pieces Adhesive Films

12. Package insert

NOTE: [96 Tests]

STORAGE

Table 1: Storage of the kit

Unopened Kit	Store at 2 – 8℃. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Standard /sample Diluent	May be stored for up to 1 month at 2 – 8℃.**
	Concentrated Biotin-Conjugate	
	Streptavidin-HRP solution	
	Biotin-Conjugate antibody Diluent	
	Streptavidin-HRP Diluent	
	Wash Buffer Concentrate 20x	
	Substrate Solution	
	Stop Solution	
	Standard	Aliquot and store for up to 1 month at -20℃. Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 – 8℃.**

**Provided this is within the expiration date of the kit

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).

2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000 μL.

3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit regents between 2℃ and 8℃. After use all reagents should be immediately returned to cold storage (2℃ to 8℃).

2. Please perform simple centrifugation to collect the liquid before use.

3. To avoid cross contamination, please use disposable pipette tips.

4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.

5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.

6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.

7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.

8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.

9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.

10. Avoid microtiter plates drying during the operation.

11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.

12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.

13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.

2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.

3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.

4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated

freeze-thaw cycles.

5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.

2. **Wash Buffer** - Dilute 10 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200 mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

3. **Standard** - Reconstitute the Standard with 1.0mL of Standard /sample Diluent. This reconstitution produces a stock solution of 1000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

    Pipette 500 µL of Standard/sample Diluent into the 500 pg/mL tube and the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 1000 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

    If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

4. Working solution of Biotin-Conjugate anti-human Adiponectin antibody: Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.

    The working solution should be used within one day after dilution.

5. Working solution of Streptavidin-HRP: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.

    The working solution should be used within one day after dilution.
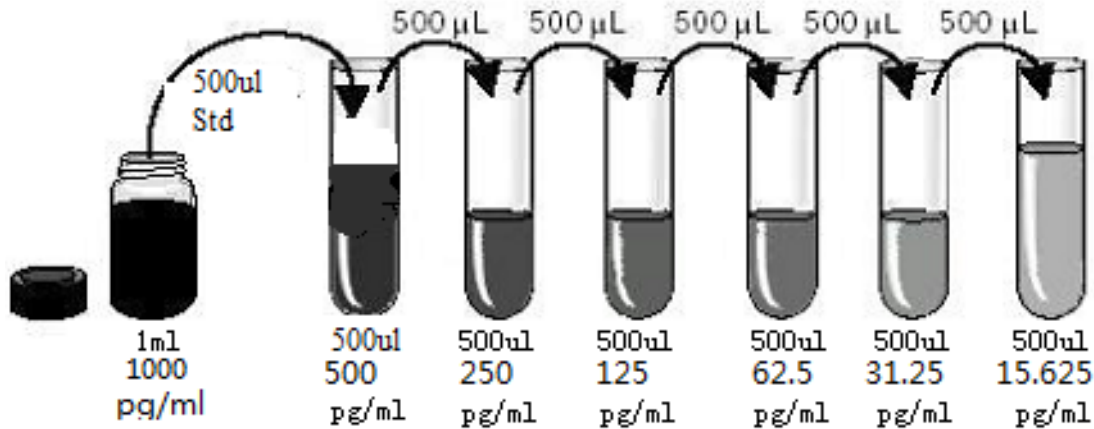
Figure 2: Preparation of Adiponectin standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.

2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.

3. Add 100 µL of Standard, control, or sample, per well. Cover with the adhesive strip provided. Incubate for 1.5 hours at 37℃.

4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (350µL) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µL of the working solution of Biotin-Conjugate to each well. Cover with a new adhesive strip and incubate 1 hour at 37℃.

6. Repeat the aspiration/wash as in step 4.

7. Add 100 µL of the working solution of Streptavidin-HRP to each well. Cover with a new adhesive strip and incubate for 30 minutes at 37℃. Avoid placing the plate in direct light.

8. Repeat the aspiration/wash as in step 4.

9. Add 100 µL of Substrate Solution to each well. Incubate for 10-20 minutes at 37℃. Avoid placing the plate in direct light.

10. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. (Optionally 630nm as the reference wave length; 610-650nm is acceptable)
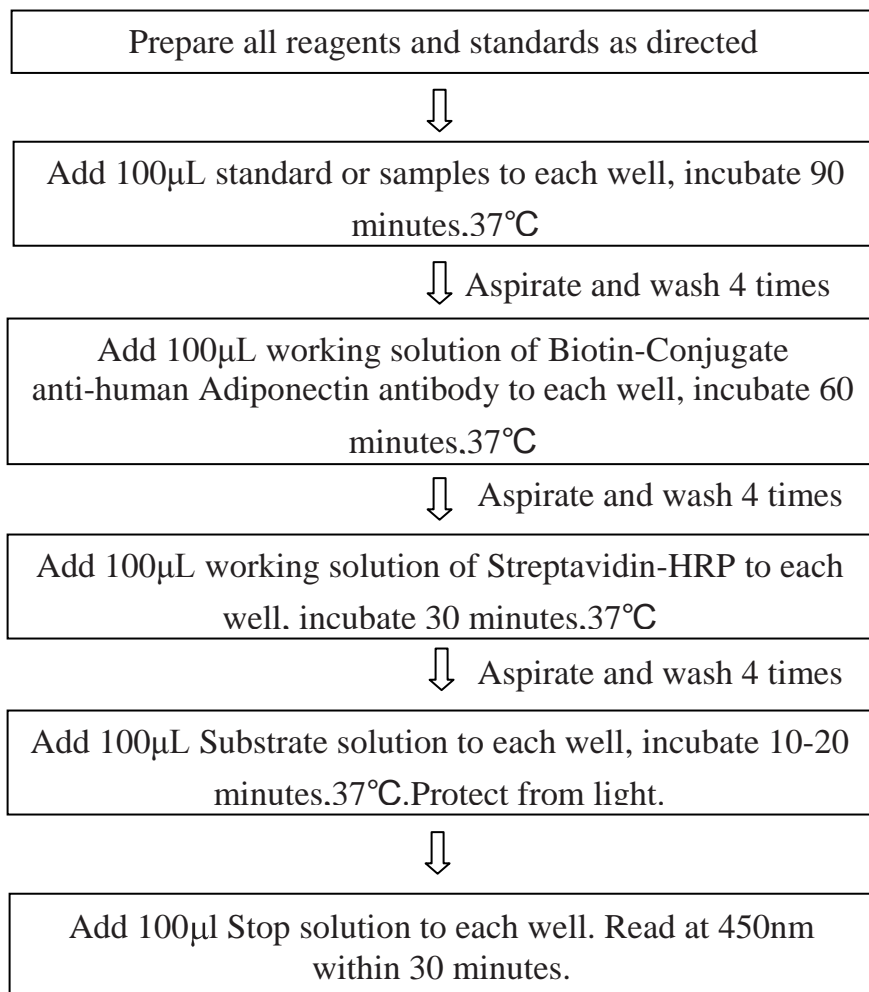
ASSAY PROCEDURE SUMMARY

Prepare all reagents and standards as directed

⇩

Add 100µL standard or samples to each well, incubate 90 minutes.37℃

⇩ Aspirate and wash 4 times

Add 100µL working solution of Biotin-Conjugate anti-human Adiponectin antibody to each well, incubate 60 minutes.37℃

⇩ Aspirate and wash 4 times

Add 100µL working solution of Streptavidin-HRP to each well. incubate 30 minutes.37℃

⇩ Aspirate and wash 4 times

Add 100µL Substrate solution to each well, incubate 10-20 minutes.37℃.Protect from light.

⇩

Add 100µl Stop solution to each well. Read at 450nm within 30 minutes.

Figure 3: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.

2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.

5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

3. The data may be linearized by plotting the log of the Adiponectin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 2:Typical data using the Adiponectin ELISA (Measuring wavelength: 450nm, Reference wavelength: 650nm)

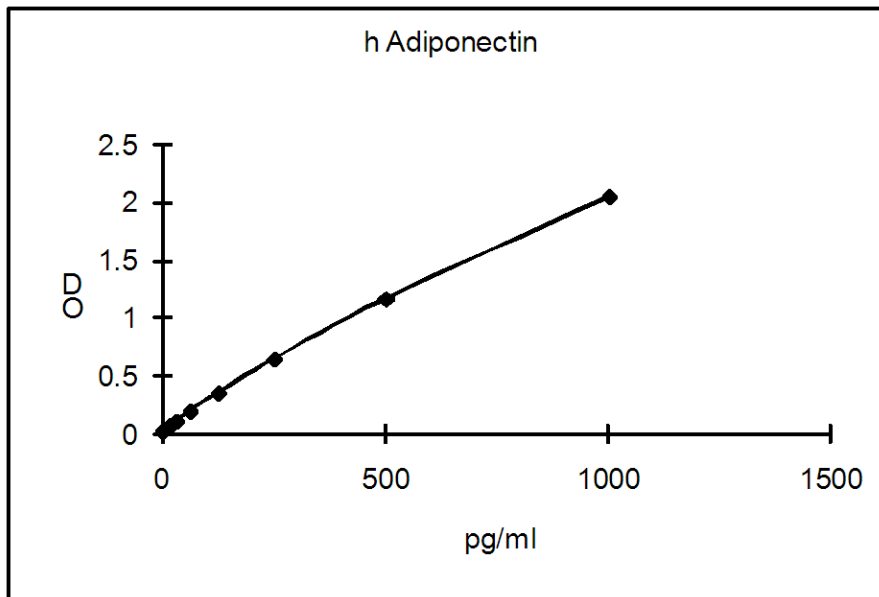| Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.022	0.019	0.021	——
15.625	0.062	0.058	0.060	0.039
31.25	0.110	0.105	0.108	0.087
62.5	0.198	0.194	0.196	0.175
125	0.349	0.340	0.345	0.324
250	0.641	0.632	0.637	0.616
500	1.162	1.154	1.158	1.137
1000	2.041	2.024	2.033	2.012

Figure 4:Representative standard curve for Adiponectin ELISA. Adiponectin was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

SENSITIVITY, SPECIFICITY AND REPEATABILITY

1. **REPEATABILITY**: The coefficient of variation of both intra-assay and inter-assay were less than 10%.
2. **SENSITIVITY**: The minimum detectable dose was 4 pg/mL.
3. **SPECIFICITY:** This assay recognizes both natural and recombinant human Adiponectin. The factors listed below were prepared at 50 ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 3: Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Other protein
4-1BB	Adiponectin	porcine TNF-α
APRIL	CD27 Ligand	rat TNF-α
BAFF/BLyS	CD30 Ligand	
CD27	Fas Ligand	
CD30 Ligand	LT-α1/β2	
CD40 Ligand	TNF-α	

REFERENCES

1. Scherer, P.E. et al. (1995) J. Biol. Chem. 270:26746.
2. Fruebis, J. et al. (2001) Proc. Natl. Acad. Sci. USA 98:2005.
3. Berg, A.H. et al. (2002) Trends Endocrinol. Metab. 13:84.
4. Arita, Y. et al. (1999) Biochem. Biophys. Res. Commun. 257:79.
5. Maeda, K. et al. (1996) Biochem. Biophys. Res. Commun. 221:286.
6. Kishore, U. and K.B. Reid (2000) Immunopharmacology 49:159.
7. Pajvani, U.B. et al. (2003) J. Biol. Chem. 278:9073.
8. Tsao, T.S. et al. (2003) J. Biol. Chem. 278:50810.
9. Hu, E. et al. (1996) J. Biol. Chem. 271:10697.
10. Yamauchi, T. et al. (2003) Nature 423:762.
11. Berg, A.H. et al. (2001) Nat. Med. 7:947.
12. Yamauchi, T. et al. (2001) Nat. Med. 7:941.
13. Tomas, E. et al. (2002) Proc. Natl. Acad. Sci. USA 99:16309.
14. Yamauchi, T. et al. (2002) Nat. Med. 8:1288.

15. Thamer, C. et al. (2002) Horm. Metab. Res. 34:646.
16. Stefan, N. et al. (2002) J. Clin. Endocrinol. Metab. 87:4652.
17. Matsubara, M. et al. (2002) Eur. J. Endocrinol. 147:173.
18. Faraj, M. et al. (2003) J. Clin. Endorinol. Metab. 88:1594.
19. Weyer, C. et al. (2001) J. Clin. Endocrinol. Metab. 86:1930.
20. Hotta, K. et al. (2000) Arterioscler. Thromb. Vasc. Biol. 20:1595.
21. Maeda, N. et al. (2001) Diabetes 50:2094.
22. Spranger, J. et al. (2003) Lancet 361:1060.
23. Matsuda, M. et al. (2002) J. Biol. Chem. 277:37487.
24. Ouchi, N. et al. (1999) Circulation 100:2473.
25. Yokota, T. et al. (2000) Blood 96:1723.
26. Ouchi, N. et al. (2001) Circulation 103:1057.

If you have any questions, please tell us!