Other ELISA kits are available from ADI (complete list at the web site)

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, E2, testosterone, progesterone etc).

Monkey: IgM, IgG, IgA, CRP

Rat: Albumin, CRP, IgG, IgM, Alpha 1 Acid glycoprotein

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha, VEGF,

Chicken: IgG, IgM, IgY, Ovalbumin

Rabbit: CRP, IgG

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Pig: Albumin, IgG, IgM,

Dog: CRP, IgG, IgM

Cat: IgG, IgM

Goat: IgG

Sheep: IgG

Turkey: IgG

KIT PROFILE

Date received: **Cat #** 100-140-ADH **Lot #** ___**Exp.** _____

Date kit opened _____ **Technician:** _____

Date used: **# Strips used** _ **# Remaining** _____

Date used: **# Strips used** _ **# Remaining** _____

Remarks _____

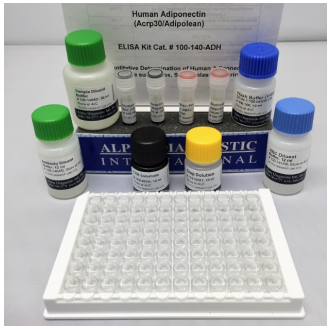
_____

Instruction Manual No. 100-140-ADH

Human Adiponectin (Acrp30/Adipolean)

ELISA Kit Cat. # 100-140-ADH

For Quantitative Determination of Human Adiponectin In cell culture supernates, Serum, plasma, & urine



For In vitro Research Use only

ALPHA DIAGNOSTIC INTERNATIONAL

India Contact:

Life Technologies (India) Pvt. Ltd. 306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA).
Ph: +91-11-42208000, 42208111, 42208222
Mobile: +91-9810521400
Fax: +91-11-42208444
Email: customerservice@lifetechindia.com
Web: www.atzlabs.com

LIFE TECH Life Technologies™ Your Molecular & Cell Technology Partner

DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Human Adiponectin ELISA KIT # 100-140-ADH

Kit Components, 96 tests	
Anti-Human Adiponectin **coated strip plate** (8 wells x 12 strips); #100-141	1 Plate
recombinant human Adiponectin **Standard**,100 ng **Lyophilized;** #100-142	2 tubes
Biotinylated-Anti-h Adiponectin Ab, 130 ul; #100-144	1 vial
Avidin-Biotin-Peroxidase complex (ABC); 130 ul; #100-145	1 vial
Sample Diluent buffer, 30 ml; #100-140SD	1 bottle
Antibody Diluent buffer, 12 ml; #100-140AD	1 bottle
ABC Diluent buffer, 12 ml; #100-140AB	1 bottle
TMB Substrate, 10 ml; #100140TM	1 bottle
Stop solution, 10 ml; 100140ST	1 bottle
Wash Buffer (100X), 10 ml; # WB-100	1 bottle
Instruction Manual; M100-140-ADH	1

Intended Use

ADI's adiponectin ELISA kit is a highly sensitive sandwich type assay for the measurement of adiponectin in serum, plasma, urine or culture supernatants. For research use only (RUO), not for diagnostic procedures.

Introduction

Adipose tissue is the largest reservoir of fuel, storing energy in the form of rapidly utilizable triglycerides. Adipocytes synthesize and store energy in periods of nutritional abundance and mobilize lipids during starvation and other times of need. In order to accomplish these complex tasks energy balance, adipocytes express many genes, including Adiponectin, involved in lipid metabolism and glucose homeostasis.

Adiponectin (also known as **Acrp30** or Adipolean) was identified as a novel adipocyte-specific synthesized and secreted protein with structural resemblance to complement factor C1q. Like adipsin, Acrp30 secretion is induced ~10-fold during adipocyte differentiation. Plasma levels are reduced in obese humans, and low levels are associated with insulin-resistance. Adiponectin (mouse 247 aa, rat/human 244 aa; chromosome 3q27) consists of a predicted NT-signal sequence 91-14 aa), followed by a 27-aa unique region, and then by 22 perfect Gly-X-Pro or Gly-X-X collagen like repeats, and a globular segment at the C-terminus. Structurally, adiponectin resembles other collagen-like and globular domain proteins (lung surfactant protein and hepatocytes mannan-binding proteins). Adiponectin is proteolytically cleaved at 104 aa to generate the globular Adiponectin (**gAcrp30**). Administration of gAcrp30 into mice fed a diet high in fat and sugar caused substantial weight loss. A marked reduction in plasma triglycerides, glucose, and free fatty acids was attributed due in part to increased fatty acid oxidation by muscle. Full length adiponectin was less potent than gAcrp30. Therefore, gAcrp30 may open new avenues to control obesity.

PERFORMANCE CHARACTERISTICS:

Intra-Assay Precision			
Sample	1	2	3
n	16	16	16
Mean(ng/ml)	13.8	37.2	67.3
Standard deviation	0.39	1.34	3.03
CV(%)	2.8	3.6	4.5

Inter -Assay Precision			
Sample	1	2	3
n	24	24	24
Mean(ng/ml)	13.1	36.8	65.7
Standard deviation	0.77	2.50	4.66
CV(%)	5.9	6.8	7.1

Range: 1.56ng/ml-100ng/ml

Sensitivity: < 60pg/ml

Specificity: Natural and recombinant human total Adiponectin

Cross-reactivity No detectable cross-reactivity with other relevant

Species Crossreactivity No detectable cross-reactivity with other relevant proteins in humans. Species crossreactivity (mouse, rat, monkey etc) has not been determined.

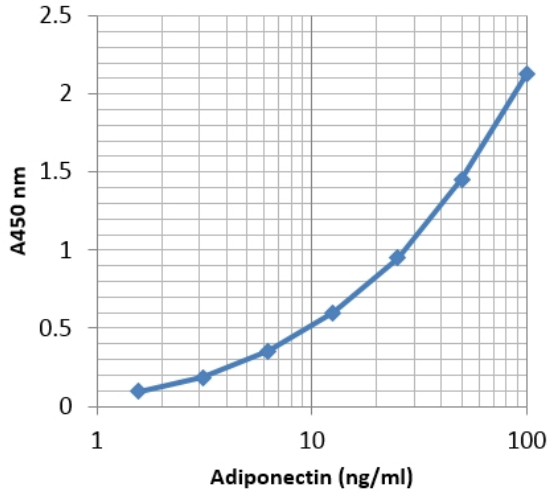
References: Yamauchi T (2002) Nature Med. 8, 1288-1295; Yokota T (200) Blood 96, 1723-1732; Takemura Y (2007) J. Clin. Invest. 117, 375-386

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	*Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Std. A** (0 ng/ml)	0.02	
B1, B2	**Std. B** (1.56 ng/ml)	0.095	
C1, C2	**Std. C** (3.12 ng/ml	0.183	
D1, D2	**Std. D** (6.25 ng/ml)	0.354	
E1, E2	**Std. E** (12.5 ng/ml)	0.6	
F1, F2	**Std. F** (25 ng/ml)	0.95	
G1, G2	**Std. G** (50 ng/ml)	1.453	
H1, H2	**Std. H** (100 ng/ml)	2.125	
	Sample 1		

*=Average duplicate values after deducting the std zero values.

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



ADI_Elisa4

A typical std. assay curve (do not use this for calculating sample values)

Plot the net A450 values of the standards and adiponectin conc using semi-log or log-log (4-point curves). Calculate the sample values from the standard cuve.

PRINCIPLE OF THE TEST

Human Adiponectin ELISA kit is based on binding of Adiponectin from samples to two antibodies, one immobilized on the microtiter well plates and biotinylated detection antibody, which then binds to streptavidin-horseradish peroxidase conjugate. After a washing step, chromogenic substrate (TMB) is added and colors developed. The enzymatic reaction (blue color) is directly proportional to the amount of adiponectin present in the sample. Adding stopping solution terminates the reaction (blue color turns yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of adiponectin in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000ul) and multi-channel pipette with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Alpha Diagnostic International human Adiponectin ELISA kit is intended for *in vitro research* use only. The reagents contain Thimerosal (0.01%) as preservative; necessary care should be taken when disposing solutions. All other precautions must be taken to handle biological material. All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid) and dispose of it accordingly.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.
TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

Sample Preparation and Storage:

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernates: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.

Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

Tissue Homogenates: Rinse tissue with PBS to remove excess blood, chopped into 1-2 mm pieces, and homogenize with a tissue homogenizer in PBS or in lysate solution (Mammal Tissue Protein Extraction Rea gent, Catalog# AR0101), lysate solution: tissue net weight = 10ml:1g(i.e. Add 10ml lysate solution to 1g tis sue). Centrifuge at approximately 5000 X g for 5 min. Assay immediately or aliquot and store homogenates at -20°C. Avoid repeated freeze-thaw cycles.

Urine: Aseptically collect the first urine of the day, micturate directly into a sterile container. Remove particular impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

DILUTION OF SAMPLES:

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

1. **High target protein concentration** (1μg-10μg /ml). The working dilution is 1:100. i.e. Add 3 μl sample into 297 μl sample diluent buffer.
2. **Medium target protein concentration** (100-1000ng/ml). The working dilution is 1:10. i.e. Add 25 μl sample into 225 μl sample diluent buffer.
3. **Low target protein concentration** (1.56-100ng/ml). The working dilution is 1:2. i.e. Add 100 μl sample to 100 μl sample diluent buffer.
4. **Very Low target protein concentration** (≤1.56ng/ml). No dilution necessary, or the working dilution is 1:2

Reagents Preparation:

1. Dilute the **wash buffer 1:100 with water**. Dilute 5ml of the stock in 500 ml water. Store at room temperature for 1 week.
2. **Preparation of biotinylated anti-human Adiponectin antibody** working solution: The solution should be prepared no more than 2 hours prior to the experiment.
3. The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
4. **Biotinylated anti-human Adiponectin antibody** should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1μl Biotinylated anti-human Adiponectin antibody to 99μl antibody diluent buffer.)
5. Preparation of **Avidin-Biotin-Peroxidase Complex (ABC)** working solution: The solution should be prepared no more than 1 hour prior to the experiment.The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume).
6. **Avidin- *Biotin-Peroxidase Complex* (ABC)** should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1μl ABC to 99μl ABC diluent buffer.)

Reconstitution of the human Adiponectin standard

Adiponectin standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of Adiponectin standard (100ng per tube) are included in each kit. Use one tube for each experiment.

1. 100ng/ml of human Adiponectin standard solution: Add 1ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.

2. 50ng/ml→1.56ng/ml of human Adiponectin standard solutions: Label 6 Eppendorf tubes with 50ng/ml, 25ng/ml,12.5ng/ml, 6.25ng/ml, 3.12ng/ml, 1.56ng/ml respectively. Aliquot 0.3ml of the sample diluent buffer into each tube. Add 0.3ml of the above 100ng/ml Adiponectin standard solution into 1st tube and mix. Transfer 0.3ml from 1st tube to 2nd tube and mix. Transfer 0.3ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 100ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of shipping under appropriate storage conditions.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

The ABC working solution and TMB color developing agent must be kept warm at **37℃ for 30 min** before use. When diluting samples and reagents, they must be mixed completely and evenly.

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1. Add 100 ul of sample diluent (blank), standards (1.56-100 ng/ml), samples (appropriate dilution) into appropriate wells in duplicate. **See "Sample Dilution on page 4" for details.** It is recommended that each human Adiponectin standard solution and each sample be measured in duplicate.

2. Cover the plate & incubate at **37℃** for **90 min**.

3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. **Do NOT** let the wells completely dry at any time.

4. Add **0.1 ml** of biotinylated anti-human Adiponectin antibody working solution into each well and incubate the plate at **37℃ for 60 min**.

5. Wash the wells with **3 times with 300 ul** of 1x wash buffer. Tap the plates over paper towels after the wash or between washing if washing manually.

6. Pipette **0.1ml** of prepared ABC working solution into each well. Mix gently. Cover the plate and incubate at **37℃ for 60 min**.

7. Wash the wells with **5 times with 300 ul** of 1x wash buffer. Tap the plates over paper towels after the wash or between washing if washing manually.

8. Add **90 ul** of TMB-substrate solution into each well. Mix gently for 5-10 secs. Cover the plate and incubate at **37℃ in dark for 15-20 minutes**. Blue color develops. **Note:** TMB solution must be at room temperature.

9. Stop the reaction by adding **100 ul of stop** solution to **all wells**. Mix gently. Blue color turns yellow.

10. Measure the absorbance at **450 nm** using an ELISA reader. Color is stable for at least 30 min after stopping.