

Human Adiponectin ELISA Procedure Summary

Total Assay Time - 225 min. (120+60+30+15)

	Allow all reagents to reach room temp.; arrange and label required # of strips. Reconstitute lyophilized Standard with 0.5 ml dH ₂ O. Dilute Sample Diluent (1:20) and Wash Buffer (1:100) with dH ₂ O. Dilute HRP-conjugate (1:100), Detection Ab (1:100), Standards (from 12-1.5 ng/ml), and serum samples (1:500-2k) with 1x Sample Diluent.
Step 1	Pipet 50 ul of 1.5-12 ng/ml Standards and diluted samples (1:500-2k) into appropriate wells. Add 50ul of 1x Sample Diluent. Mix gently, cover the plate and incubate for 120 min at room temp
Step 2	Aspirate and wash 3 times with 1x wash solution. Dispense 100 ul of 1x Detection Antibody to each well. Mix gently, cover the plate and incubate for 60 min at room temp.
Step 3	Aspirate and wash 3 times with 1x wash solution. Dispense 100 ul of 1x Streptavidin-HRP conjugate to each well. Mix gently, cover the plate and incubate for 30 min at room temp.
Step 4	Aspirate and wash 3 times with 1x wash solution. Dispense 100 ul of TMB Substrate . Mix gently, cover the plate and incubate for 15 min at room temp. Blue color develops.
Step 5	Pipette 100 ul Stop Solution (1% H ₂ SO ₄) into each well. Blue color turns yellow. Measure absorbance at 450 nm.

Human Serum Adiponectin (Acrp30/Adipolean)

For Quantitative Determination of Adiponectin In Human Serum

CHECK LIST (Check each box after completing each of the above steps)

	Step 1	Step2	Step3	Step4	Step5
Start time					
End Time					

Date kit opened _____ **Technician:** _____

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

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

Remarks _____

Human Adiponectin ELISA KIT # 100-140-ADH

Kit Components, 96 tests	Cat #
Anti-Human Acrp30 coated strip plate (8 wells x 12 strips)	100-141
Human Acrp30 Std. A (0 ng/ml), 0.500 ml	100-142A
Lyophilized Human Acrp30 Std. E (6 ng), 2 vials Reconstitute with 0.5 ml dH ₂ O	100-142B
Biotin-Anti-hAcrp30 Detection Ab, (100x), 0.12 ml	100-143
(20x) Sample Diluent, 10ml	SD-20
Wash Buffer (100X), 10 ml	WB-100
(100x) Streptavidin-HRP conjugate, 0.12ml	S-HRP100
TMB Substrate, 12 ml	80091
Stop solution, 12 ml	80101
Instruction Manual	100-140-ADH

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 adiponectin, 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.

ADI's adiponectin ELISA kit is a highly sensitive sandwich type assay for the measurement of adiponectin in serum. The assay can be adapted for other biological fluids such as plasma, urine, culture medium etc.

PERFORMANCE CHARACTERISTICS

1. Detection limit- Based on 4 replicate determinations of the zero standards, the minimum Adiponectin concentration detectable using this assay is ~ 0.8 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

2. Expected Values: A limited testing of 20 adult human serum samples values of 1.8 - 5.25 ug/ml (average 3.08 ug/ml).

3. Specificity: This kit is specific to full length human adiponectin (Acrp30) and does not show any significant reactivity to gAcrp30 other human serum proteins.

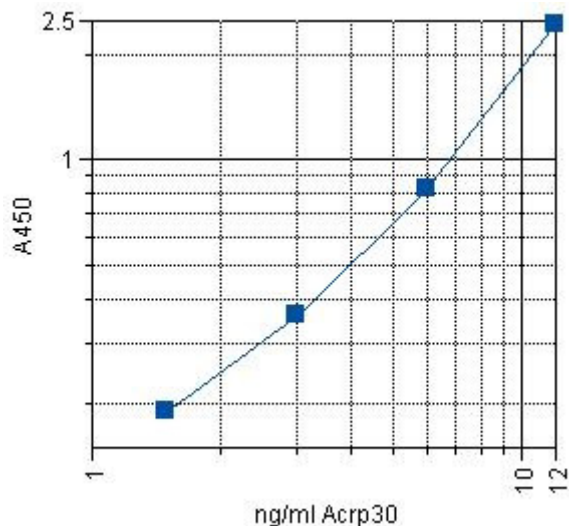
4. Species Crossreactivity -Cross reactivity was tested with the following serum proteins at 8 ng/ml: Mouse Acrp30, Human gAcrp30, and Mouse gAcrp30 all had less than 1% cross reactivity. Cross reactivity was tested with the following animal serum at 1:100 dilution: Monkey serum had significant reactivity(>100%). Mouse, Rat, Guinea Pig, Bovine, Sheep, and Goat serum (full length Acrp30 or gAcrp30) all had less than 1% crossreactivity.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	*Mean A ₄₅₀ nm	Calculated Conc'n
A1, A2	Std. A (0 ng/ml)	0.0	
B1, B2	Std. B (1.5 ng/ml)	0.19	
C1, C2	Std. C (3 ng/ml)	0.36	
D1, D2	Std. D (6 ng/ml)	0.83	
E1, E2	Std. E (12 ng/ml)	2.45	
	Sample 1 (1:1k)	0.50	(4 ng/ml) Adjusted for sample dilution 4.0 ug/ml

*=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.



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

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

Standards and Sample Diluent contain Proclin 300 (0.05%, v/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid and Proclin 300, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum. It is also possible to use plasma for testing.

Reagent Preparation

1. Dilute the 20X Sample Diluent 1:20 with dH₂O (e.g., 5ml diluent + 95ml dH₂O). Prepare only the required reagent; 100 ml for a full plate assay. Store diluted solution at 2-8°C for up to a week.
2. Dilute Standard E (6ng) with 500ul of dH₂O to equal 12ng/ml Acrp30. Vortex well and let stand for 15 minutes before use. The reconstituted and diluted standards must be used within 24 hours.
3. Dilute the Wash Buffer 1:100 with dH₂O (e.g., 5ml of stock + 495 ml dH₂O). Store at room temperature for 1 week.
4. Dilute the Detection Ab (1:100) using the 1x Sample Diluent (100 ul + 9.9 ml Sample Diluent). Prepare 10 ml for a full plate assay.
5. Dilute the Streptavidin-HRP (1:100) using the 1x Sample Diluent (100 ul + 9.9 ml Sample Diluent). Prepare 10 ml for a full plate assay.

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 3 months from the date of shipping under appropriate storage conditions.

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

1. Dilute standards using the following dilution scheme:

Initial Conc'n	Amount of Standard	Amount of Diluent	Total Volume	Final Conc'n
12 ng/ml	250ul	250ul	500ul	6 ng/ml
6 ng/ml	250ul	250ul	500ul	3 ng/ml
3 ng/ml	250ul	250ul	500ul	1.5 ng/ml

- Dilute human serum samples 1:1000 using 1x Sample Diluent. Some samples may have to be diluted more or less but 1:1k should bring most normal samples to within the testing range.

	Sample	Diluent	Total Volume	Dilution Factor
Step 1	5 ul undiluted sample	495ul	500 ul	1:100
Step 2	20 ul of 1:100	180 ul	200 ul	1:1000

Note: It is possible to adjust the sample dilution to make 1:500 and a 1:2000 dilution, we recommend testing them both.

- Label or mark the microtiter well strips to be used on the plate.
- Dispense 200-300 ul of wash buffer to all wells. Let stand for 5 to 15 minutes, then discard or aspirate the solution. The step should be done just before adding the samples, do not allow the wells to dry at any time during the assay.
- Pipet **50 ul standards** and diluted samples into appropriate wells.
- Note:** for ease of loading samples it is recommended that a second **uncoated** microwell plate should be used keeping diluted samples. This enables standards or samples to be transferred quickly to the ELISA plate using multichannel pipette.
- Add **50ul** of 1x Sample Diluent to all wells. Mix gently, cover the plate and incubate at room temperature for **120 minutes**.
- Wash the wells with **3 times with 300 ul** of 1x wash buffer.
- Pipette 100 ul** of 1x adiponectin Detection antibody into each well. Mix gently. Cover the plate and incubate for **60 minutes** at room temp. **Note:** the detection solution must be at room temp.

- Wash the wells with **3 times with 300 ul** of 1x wash buffer.

- Pipette **100 ul of 1x Streptavidin-HRP-enzyme** into **each well**. **Mix gently**. Cover the plate and incubate for **30 minutes** at room temperature. **Note:** the conjugate solution must be at room temperature.
- Aspirate and wash the wells **3 times** with 1x wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
- Add **100 ul** of HRP-substrate soln. (TMB) into each well. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature. Blue color develops. **Note:** TMB solution must be at room temperature.
- Stop the reaction by adding **100 ul of stop** solution to **all wells**. Mix gently. Blue color turns yellow.
- Measure the absorbance at **450 nm** using an ELISA reader. Color is stable for at least 30 minutes after stopping.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 2-8°C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.

DILUTION OF SAMPLES

Samples containing more than **12 ng/ml** human Adiponectin should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Subtract the absorbance of the zero standard from the mean absorbance values of standards and samples. Draw the standard curve on log graph paper by plotting net absorbance values of standards against appropriate adiponectin concentrations. Read off the adiponectin concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:1K then the values must be multiplied by 1,000 with the results expressed as ug/ml.