

BioAim Scientific Inc

Human Adiponectin EasyTestTM ELISA Kit

Cat.No: 1010001

Instruction Manual

For research use only

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I. INTRODUCTION

Adiponectin, also known as Acrp30, is an adipocyte-specific, secreted protein with potential roles in glucose and lipid homeostasis. 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. Adiponectin is induced during adipocyte differentiation and its secretion is stimulated by insulin. Two receptors for Adiponectin, termed AdipoR1 and AdipoR2, have been cloned. 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. This is likely due to insulin-sensitizing effects involving Adiponectin regulation of triglyceride metabolism. 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 PPAR γ . Adiponectin may also play anti-atherogenic and anti-inflammatory roles. Adiponectin plasma levels are decreased in patients with coronary artery disease. Adiponectin inhibits endothelial cell expression of adhesion molecules in vitro, suppressing the attachment of monocytes. In addition, Adiponectin negatively regulates myelomonocytic progenitor cell growth and TNF- α production in macrophages.

The BioAim Human Adiponectin EasyTestTM ELISA kit can quantitatively measure Adiponectin in human serum or plasma. It is a simple and rapid technology for the quantitation of antigen in a range of sample matrices. The whole process takes less than 2.5 hours with high accuracy and precision. EasyTestTM ELISA is faster and easier to perform than standard format ELISA with less reagent handling and fewer pipetting steps.

II. MATERIALS SUPPLIED

1. Human Adiponectin Microplate: 96 breakable wells (12strips x 8wells) coated with anti-human Adiponectin.
2. 20x Wash Buffer Concentrate: 1 Vial, 25 ml.
3. 5x Assay Diluent: 1vial, 15 ml.
4. Standards: 2 vials, recombinant human Adiponectin.
5. BioAim human Adiponectin Mix: 9µl/vial, 4 vials.
6. TBM Substrate solution: 1 Vial, 12 ml.
7. Stop Solution: 1 Vial, 8 ml of 0.2 M sulfuric acid.

III. STORAGE

1. The kit can be stored for up to 6 months at 2° to 8°C from the date of shipment.
2. Standard can be stored at -20 °C or -80 °C. Use freshly prepared standard within 12hours (stored at 2~8°C).
3. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8 °C. Return unused strip to the pouch containing desiccant pack, reseal along entire edge and keep in 2~8 °C.
4. Avoid repeated freeze-thaw cycles.

IV. ADDITIONAL MATERIALS REQUIRED

1. Distilled or deionized water.
2. Precision pipettes, with disposable plastic tips.
3. Beakers, flasks, cylinders necessary for preparation of reagents.
4. Microplate washing device (multichannel pipette or automated microplate washer).
5. Microplate shaker.
6. Microplate reader capable of reading at 450 nm.

V. PRECAUTIONS

1. All reagents must be at room temperature (18 °C to 25 °C) before running assay.
2. Do not mix or substitute reagents with those from other lots or other sources.
3. Do not use kit reagents beyond expiration date on label.
4. Do not expose kit reagents to strong light during storage or incubation.
5. Use disposable pipette tips for each transfer to avoid microbial contamination or cross contamination of reagents.
6. Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results.
7. Avoid contact of stop solution with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
8. Do not use TMB substrate solution if it has begun to turn blue.
9. Do not expose bleach to work area during actual test procedure because of potential interference with enzyme activity.

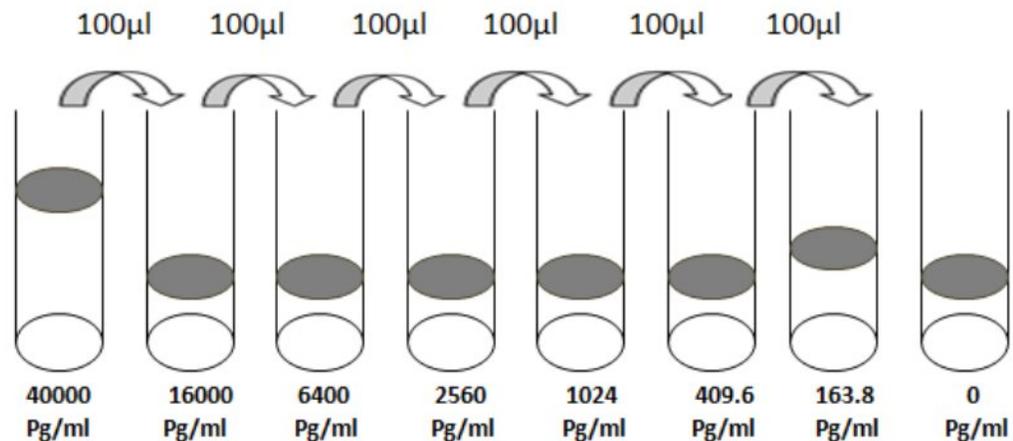
VI. REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (18~25°C) before use.
2. **Assay diluent**
Dilute the concentrated assay diluent 1:5 with distilled water (e.g. 10ml plus 40ml).
3. **Wash Buffer**
Dilute the concentrated wash buffer 1:20 with distilled water (e.g. 20ml plus 380ml).
4. **Sample**
Levels of the target protein may vary among different specimens. Optimal dilution factors for each sample must be determined by the investigator.

The dilution scheme is only suggestion: the recommended dilution for serum and plasma is 1: 1000.

5. Standard

- Briefly spin standard vial before use. Add 500 μl 1x Assay Diluent to prepare a 100ng/ml standard. Gently vortex to mix.
- Take 200 μl Adiponectin standard into a tube; then add 300 μl 1x Assay Diluent to prepare a 40000 pg/ml stock standard solution.
- Add 150 μl 1x Assay Diluent to 7 tubes. Label as 16000pg/ml, 6400pg/ml, 2560pg/ml, 1024pg/ml, 409.6pg/ml, 163.8pg/ml and the last tube with 1x assay diluent is the blank as 0pg/ml.
- Perform serial dilutions by adding 100 μl of each standard to the next tube and vortexing between each transfer (see figure below).



6. BioAim human Adiponectin Mix

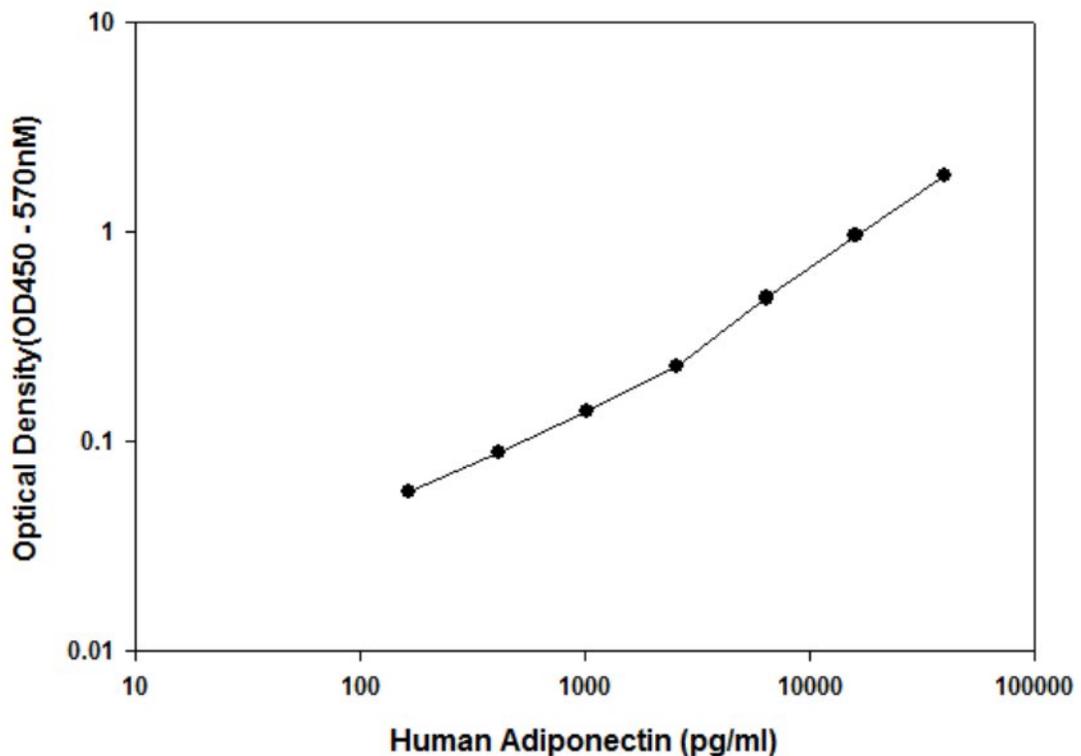
Within 15 minutes prior to use, briefly spin the vial. Add 1490 μl of 1x Assay diluent to the vial and mix by pipetting. A vial mix can be used for around 30 wells.

VII. ASSAY PROCEDURE

1. All reagents must be brought to room temperature (18-25°C) prior to use. Place the required number of microwells in the holder. It is recommended that all samples, standards, and blanks be run in duplicate.
2. Add 50 µl of 1x Assay Diluent into the blank wells.
3. Add 50 µl of each standard (*see reagent preparation step 5*) and samples into the designated wells. Gently shake/tap the plate for 5 seconds to mix.
4. Add 50 µl of BioAim Adiponectin Mix into all wells, including the blank wells.
5. Cover wells with plate sealer and incubate at room temperature (18~25°C) for 2 hour with gentle shaking.
6. Decant or aspirate contents of wells. Wash wells by filling with at least 300 µl/well prepared wash buffer followed by decanting/aspirating. Soak wells in wash buffer for 30 seconds to 1 minute for each wash. Repeat wash 4 times for a total of 5 washes. After the last wash, blot plate on absorbent paper to remove residual buffer. Thorough washing at this step is very important, complete removal of liquid is required for proper performance.
7. Pipette 100 µl of TMB Substrate Solution to each well. Incubate plate for 15 minutes at room temperature in the dark with gentle shaking.
8. Add 50 µl of stop solution to each well.
9. Read absorbance at 450nm within 30 minutes of stopping reaction. If wavelength correction is available, subtract the optical density readings at 570nm from readings at 450nm.

VIII. CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of duplicate standards, samples and controls. Subtract the average zero standard optical density.
2. Create a standard curve by plotting the mean absorbance for each standard concentration on the ordinate against the Adiponectin concentration on log-log graph paper or using Sigma plot software. Draw a best fit curve through the points of the graph.
3. To determine the concentration of circulating Adiponectin for each sample, first find the mean absorbance value on the ordinate and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the abscissa and read the corresponding Adiponectin concentration.
4. A representative standard curve is shown below. This standard curve is for demonstration only. A standard curve must be run with each assay by operator.



IX. PERFORMANCE

A. Sensitivity

The minimum detectable dose of Adiponectin was determined to be 18pg/ml. This is defined as two standard deviations above the mean optical density of 20 replicates of the zero standards.

B. Recovery

Recovery was determined by spiking various levels of Human Adiponectin into the diluted sample types listed below. Mean recoveries are as follows:

Sample Type	Average % recovery	Range %
Serum	102	94-105
Plasma	106	97-112

C. Linearity

Sample	Dilution	% of expected
Seum	1:2	98
	1:4	101
	1:8	90
Plasma	1:2	97
	1:4	102
	1:8	89

D. Specificity

No cross-reactivity was identified with the following cytokines: BDNF, IL-1 beta, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-9, IP-10, G-CSF, GM-CSF, IFN-gamma, Leptin, MCP-1, PDGF, RANTES, SCF, TGF-beta, TIMP-2, TNF-alpha, TNF-beta, and VEGF.

E. Reproducibility

Intra-Assay CV%: <10%

Inter-Assay CV%: <15%

X. REFERENCES

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XI. TROUBLESHOOTING

Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting 2. Improper standard dilution	1. check pipettes; 2. Ensure briefly spin the vial of standard, take the right amount to dilution.
2. Low signal	1. Too brief incubation time 2. Inadequate reagent volumes or improper dilution	1. ensure adequate incubation time; 2. Check pipettes and ensure corrected preparation.
3. Large CV	Inaccurate pipetting	1. Check pipettes; 2. Accurately perform each step.
4. High background	1. Plate is insufficiently washed; 2. Wash buffer contamination	1. Follow the manual correctly; if using a plate washer, check that all ports are working functionally; 2. Prepare fresh buffer.
5. Low sensitivity	1. ELISA kit improper storage 2. Stop solution	1. Follow the manual to store each component correctly; 2. Add enough stop solution to each well.

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