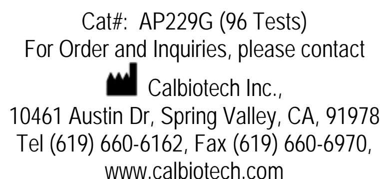
LIMITATIONS OF THE TEST

1. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

REFERENCES

1. Meier U and Gressner AM (2004) Endocrine Regulation of Energy metabolism: Review of Pathobiochemical and Clinical Aspects of Leptin, Ghrelin, Adiponectin, and Resistin. *Clin Chem* 50:1511-1525
2. Duntas LH, Popovic V and Panotopoulos G (2004) Adiponectin: Novelties in Metabolism and Hormonal Regulation. *Nutr Neurosci* 7:195-200
3. Lara Castro C, Luo N, Wallace P, Klein RL and Grvey T (2006) Adiponectin multimeric Complexes and the Metabolic Syndrome Trait Cluster. *Diabetes* 55:249-259

2014-07-07



Cat#: AP229G (96 Tests)
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Adiponectin ELISA

Catalog No. AP229G (96 Tests)

INTENDED USE

The Calbiotech, Inc (CBI) Adiponectin ELISA kit is used for the quantitative measurement of Adiponectin in human serum or plasma. For research use only.

CLINICAL UTILITY

Adiponectin is an adipocyte-secreted hormone, consisting of 244 amino acids with a molecular weight of approximately 30kDa (28-30kDa). It is one of the most abundant proteins in human blood, with circulating concentrations of 0.5-30 µl/mL, which accounts for approximately 0.01% of total plasma protein [1]. Adiponectin concentrations is reversely associated with type 2 diabetes, coronary artery disease and obesity, all together called the metabolic syndrome. Adiponectin decreases blood glucose and free fatty acid serum concentrations and increases insulin sensitivity [2]. Adiponectin has been shown to have anti-inflammatory effects [1]. However, recent studies indicate that Adiponectin may not be present in circulation as monomers or isolated globular forms, but rather in multimeric structures. The studies have shown that the dominant forms of Adiponectin that circulates in human blood are hexamers (LMW) and lager oligomers (HMW) [3,12-14]. The LMW Adiponectin levels does not seem to differ between insulin sensitive- and insulin resistant subjects, nor does LMW Adiponectin differ between men and women. The increased levels of total Adiponectin in insulin sensitive subjects and women were caused by increased amounts of HMW Adiponectin. Both total and HMW Adiponectin showed significant differences between the insulin sensitive- andinsulin resistant subjects.

PRINCIPLE OF THE TEST

The CBI Adiponectin ELISA is a solid phase sandwich enzyme immunoassay. Two monoclonal antibodies are directed against separate antigenic determinants on the Adiponectin molecule. During incubation, Adiponectin in the sample react with anti-Adiponectin antibodies bound to microwell plate wells and anti-Adiponectin antibodies bound to HRP. A simple washing step removes unbound enzyme labeled antibody. The bound conjugate is detected by reaction with 3,3'5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is red spectrophotometrically.

MATERIALS PROVIDED	96 Tests
Microwells coated with Anti-Adiponectin Ab	12x8x1
Adiponectin Calibrators Set (6 Vials)	0.25 ml
Enzyme Conjugate	12 ml
Wash Buffer	25 ml
TMB Substrate	12ml
Stop solution	12 ml

MATERIALS NOT PROVIDED

1. Distilled or deionized water
2. precision pipettes
3. Disposable pipette tips
4. Micortiter well reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

STORAGE AND STABILITY

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials: The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. This test kit is designed for research use only.
3. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
4. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
5. It is recommended that standards, control and serum samples be run in duplicate.
6. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8° C) for 5 days. If storage time exceeds 5 days, store frozen at (-20° C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.

Do not use grossly lipemic specimens.

REAGENTS PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution to 1000 ml with distilled deionized water In a suitable storage container. Store at room temperature (18-26° C).
2. **Working Substrate Solution**
   Pour the content of the vial labeled Solution 'A' into the vial labeled 'B'. Mix and labeled accordingly.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26° C).

1. Format the microplate wells for each serum reference, control and patient specimen to be assayed in duplicate. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8° C.
2. Pipette 25µl of the appropriate serum reference, control or specimen into the assigned well.
3. Add 100µl of the enzyme conjugate reagent to each well. It is very important to dispense all reagents close to the bottom of the coated well.
4. Incubate 60 minutes at room temperature on a plate shaker set to 600 RPM.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes.
7. Add 100µl of working TMB substrate solution to all wells. Always add reagents in the same order to minimize reaction time differences between wells. Don't shake the plate after substrate addition.
8. Incubate at room temperature for fifteen (15) minutes.
9. Add 50µl of stop solution to each well and mix gently for 30 seconds. Always add reagents in the same order to minimize reaction time differences between wells.
10. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. The results should be read within fifteen (15) minutes of adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

1. Check Adiponectin standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the Adiponectin standards (vertical axis) versus the Adiponectin standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve

	OD 450 nm	Conc. µIU/mL
Standard 1	.025	0
Standard 2	.096	3
Standard 3	.223	6
Standard 4	.497	15
Standard 5	1.194	35
Standard 6	2.095	70