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# Adiponectin ELISA Assay Kit

Catalog Number:

ADP31-K01 (1 x 96 wells)

*For Research Use Only. Not for use in diagnostic procedures.*

*v. 1.0*

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## **INTENDED USE**

The Eagle Biosciences Adiponectin ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of Adiponectin in human serum and plasma by an enzyme immunoassay method. The Eagle Biosciences Adiponectin ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

## **INTRODUCTION**

Adiponectin is a hormone that modulates glucose regulation and fatty acid oxidation. It is secreted from adipose tissue and placenta into the bloodstream and represents 0.01% of all plasma protein. Adiponectin increases insulin sensitivity and decreases plasma glucose by increasing tissue fat oxidation. Adiponectin concentrations correlate negatively with glucose, insulin and triglycerides (TG) concentrations, liver fat content and body mass index and positively with high density lipoprotein cholesterol levels, hepatic insulin sensitivity and insulin stimulated glucose disposal. Adiponectin levels decrease in patients with type 2 diabetes and in patients with coronary heart disease.

Adiponectin could be used as a marker for:

- Energy metabolism and body weight regulation.
- Metabolic syndrome.
- Type 2 diabetes.
- Coronary artery disease.
- Atherosclerosis.

## **PRINCIPLE OF THE ASSAY**

The principle of the adiponectin ELISA is a two-step sandwich enzyme immunoassay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for adiponectin is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of adiponectin is conjugated to biotin. During the first step, adiponectin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Unbound biotinylated antibody is removed by a washing. In the second step, streptavidin-HRP is added, which binds specifically to bound biotinylated antibody. Unbound streptavidin-HRP is removed by washing. Next, the enzyme substrate (TMB) is added. The colour intensity of the enzymatic reaction is directly proportional to the concentration of adiponectin. The enzymatic reaction is terminated by the addition of stopping solution. The absorbance is measured on a microplate reader at 450 nm. The concentration of adiponectin in samples and controls can be calculated from a plot of the standard curve, either graphically or by using immunoassay software.

## **PROCEDURAL CAUTIONS AND WARNINGS**

1. This kit is for research use only.
2. Practice good laboratory practices when handling kit reagents.

This includes:

- Do not pipette by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Wear protective clothing and disposable gloves when handling the specimens and kit reagents.
- Wash hands thoroughly after performing the test.



- Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
  4. Avoid microbial contamination of reagents.
  5. A calibrator curve must be established for every run.
  6. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
  7. The controls (included in kit) should be included in every run and fall within the acceptable ranges, as stated in the quality control certificate.
  8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
  9. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
  10. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
  11. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
  12. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
  13. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
  14. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
  15. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
  16. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

## **LIMITATIONS**

1. All the reagents within the kit are calibrated for the direct determination of adiponectin in human serum and plasma. The kit is not calibrated for the determination of adiponectin in other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum or plasma.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only the dilution buffer provided with the kit may be used to dilute serum or plasma samples. The use of any other reagent may lead to false results. Note: The dilution buffer must be prepared before use.
5. Samples must be diluted immediately before running the assay.
6. This kit is intended for research use only and should not be used for diagnostic procedures.



## **SAFETY CAUTIONS AND WARNINGS**

### **POTENTIAL BIOHAZARDOUS MATERIAL**

All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices.

### **CHEMICAL HAZARDS**

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

### **SPECIMEN COLLECTION AND STORAGE**

Approximately 0.05 mL of serum or plasma is required per duplicate determination.

Serum: Collect 2-3 mL of blood into an appropriately labelled tube and allow it to clot.

Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

Plasma: Collect 2-3 mL of blood into EDTA plasma tubes. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date.

### **REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED**

1. Precision pipettes to dispense 10, 20, 50, 100, and 150  $\mu$ L
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate washer (recommended)
6. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater\* (see assay procedure step 10)

### **REAGENTS PROVIDED**

**1. Anti-Adiponectin Monoclonal Antibody-Coated Break-Apart Well Microplate — Ready To Use**

Contents: One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

**2. Monoclonal Anti-Adiponectin-Biotin Conjugate — Ready to Use**

Contents: One bottle containing a monoclonal anti-adiponectin antibody conjugated to biotin, in a stabilizing buffer with a non-mercury preservative.

Volume: 13 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.

**3. Streptavidin-Horseradish Peroxidase (HRP) Conjugate — Ready to Use**

Contents: One bottle containing Streptavidin-HRP conjugate in a stabilizing buffer with a non-mercury preservative.

Volume: 13 mL/bottle

Storage: Refrigerate at 2–8°C

Stability: 12 months or as indicated on label.



4. **Adiponectin Calibrators** — Ready To Use
  - Contents: Six vials containing adiponectin in a buffer with a non-mercury preservative. Calibrators were prepared by spiking matrix with a defined quantity of adiponectin.
  
  - Calibrator concentrations\*: 0, 2, 5, 10, 25 and 50 ng/mL
  - \* Approximate value – please refer to vial labels for exact concentrations.
  
  - Volume: Calibrators A-F: 1 mL/vial
  - Storage: Refrigerate at 2–8°C.
  - Stability: 12 months in unopened vials or as indicated on label.
  
5. **Adiponectin Controls** — Ready to Use
  - Contents: Two vials containing adiponectin in a buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of adiponectin. Refer to vial labels for the acceptable range.
  
  - Volume: 1 mL/vial
  - Storage: Refrigerate at 2–8°C
  - Stability: 12 months in unopened vials or as indicated on label.
  
6. **Dilution Buffer Concentrate** — Requires Preparation X2
  - Contents: One bottle containing buffer with a non-mercury preservative.
  - Volume: 50 mL/bottle
  - Storage: Refrigerate at 2–8°C
  - Stability: 12 months or as indicated on label.
  - Preparation: Dilute the dilution buffer concentrate 1:2 in distilled or deionized water to prepare the working dilution buffer. Example: To prepare 10 mL of working dilution buffer, add 5 mL of water to 5 mL of dilution buffer concentrate.
  
7. **Wash Buffer Concentrate** — Requires Preparation x10
  - Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
  - Volume: 50 mL/bottle
  - Storage: Refrigerate at 2–8°C
  - Stability: 12 months or as indicated on label.
  - Preparation: Dilute the wash buffer concentrate 1:10 in distilled or deionized water to prepare the working wash buffer. If one whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.
  
8. **TMB Substrate** — Ready To Use
  - Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
  - Volume: 16 mL/bottle
  - Storage: Refrigerate at 2–8°C
  - Stability: 12 months or as indicated on label.
  
9. **Stopping Solution** — Ready To Use
  - Contents: One bottle containing 1M sulfuric acid.
  - Volume: 6 mL/bottle
  - Storage: Refrigerate at 2–8°C
  - Stability: 12 months or as indicated on label.



## PREPARATION OF SAMPLES

1. Prepare the required amount of working dilution buffer (see Reagents Provided section, Dilution Buffer), which will be used to dilute the samples with before using in the assay.
2. Dilute the serum and plasma samples 1:1000 in the working dilution buffer before starting the assay. It is recommended to perform the dilution in two steps, as indicated below:

**Dilution A (25X):** Add 20  $\mu\text{L}$  of sample to 480  $\mu\text{L}$  of working dilution buffer. Mix well, avoiding the formation of foam.

**Dilution B (40X):** Add 10  $\mu\text{L}$  of Dilution A to 390  $\mu\text{L}$  of working dilution buffer to prepare the final dilution (1:1000). Mix well, avoiding the formation of foam.

## ASSAY PROCEDURE

Specimen Pretreatment: None.

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature mix gently by inversion. Dilute all samples 1:1000 in the working dilution buffer before use (see preparation of samples section). Prepare the working wash buffer (see wash buffer concentrate under reagents provided section).
2. Remove the required number of well strips from the microplate and assemble into a plate frame. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 50  $\mu\text{L}$  of each calibrator, control and diluted (1000x) serum or plasma sample into correspondingly labelled wells in duplicate. Note: Do not dilute the calibrators or kit controls.
4. Pipette 100  $\mu\text{L}$  of the monoclonal anti-adiponectin-biotin conjugate into each well (the use of a multichannel pipette is recommended).
5. Incubate on a plate shaker (~200 rpm on a linear shaker or ~600 rpm on an orbital shaker) for 60 minutes at room temperature.
6. Wash the wells 3 times each time with 300  $\mu\text{L}$  of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of a washer is recommended.) The performance of this assay is markedly influenced by the correct execution of the washing procedure.
7. Pipette 100  $\mu\text{L}$  of the streptavidin-HRP conjugate into each well (the use of a multichannel pipette is recommended).
8. Incubate on a plate shaker (~ 200 rpm on a linear shaker or ~600 rpm on an orbital shaker) for 30 minutes at room temperature.
9. Wash the wells 3 times using the same procedure as in step 6.
10. Pipette 150  $\mu\text{L}$  of the TMB substrate into each well at timed intervals (the use of a multichannel pipette is recommended).
11. Incubate on a plate shaker (~200 rpm on a linear shaker or ~600 rpm on an orbital shaker) for 10 to 15 minutes at room temperature or until calibrator F attains dark blue colour for desired OD).
12. Pipette 50  $\mu\text{L}$  of stopping solution into each well at the same timed intervals as in step 10. Mix thoroughly by gently tapping the plate.
13. Measure the absorbance at 450 nm in all wells with a microplate reader, within 20 minutes after addition of the stopping solution.



## CALCULATIONS

1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the serum and plasma samples directly off the calibrator curve.
5. The measured concentration of samples calculated from the standard curve must be multiplied by the dilution factor of 1000. Example: 15 ng/mL (from standard curve) x 1000 (dilution factor for serum and plasma samples) = 15 µg/mL.
6. If a sample reads more than calibrator F then dilute the original 1000x diluted sample with working dilution buffer at a dilution of no more than 1:8. The result obtained must be multiplied by the dilution factor.
- 7.

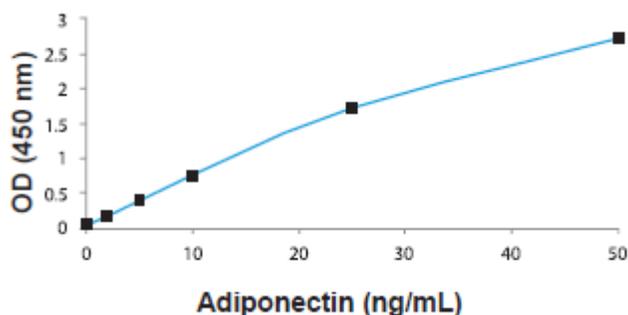
## TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD	Adiponectin (ng/mL)
A	0.04	0
B	0.16	2
C	0.38	5
D	0.75	10
E	1.71	25
F	2.72	50
Unknown	0.41	5.42

## TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.



## PERFORMANCE CHARACTERISTICS

### SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 60 samples of the blank and a low value sample and it was calculated as follows:

$$\text{LoD} = \mu_B + 1.645\sigma_B + 1.645\sigma_S,$$

where  $\sigma_B$  and  $\sigma_S$  are the standard deviation of the blank and low value sample and  $\mu_B$  is the mean value of the blank.

LoD = 0.06 ng/mL of Adiponectin



### SPECIFICITY (CROSS-REACTIVITY)

The blank was spiked separately with 100 ng/mL of human leptin, human resistin, human TNF- $\alpha$  and IL-6 and 10 ng/mL of C-peptide. The signal obtained for each was compared to the signal of adiponectin at 10 ng/mL.

$$\% \text{ Cross-Reactivity} = (\text{Signal of substance tested} / \text{Signal of Adiponectin at 10 ng/mL}) * 100$$

Analyte	Concentration (ng/mL)	% Cross-Reactivity
Leptin	100	0
TNF-a	100	0
IL-6	100	0.9
Resistin	100	0.1
C-peptide	10	0

### INTERFERENCE

Interference testing was performed according to CLSI guideline EP7-A2. Serum samples with varying levels of adiponectin were spiked with potential interfering substances at recommended levels and analyzed. Results were compared to the same serum samples with no extra substances added to calculate the % interference. The following substances were tested and did not show significant interference in the assay: hemoglobin up to 0.25 g/L, bilirubin conjugated and free up to 85  $\mu$ M, triglycerides up to 5.5 mg/mL and human serum albumin up to 60 g/L.

### INTRA-ASSAY PRECISION

Three samples were assayed 20 times each on the same calibrator curve. The results (in  $\mu$ g/mL) are tabulated below:

Sample	Mean	SD	CV %
1	6.59	0.36	5.5
2	11.92	0.55	4.6
3	36.82	2.75	7.5

### INTER-ASSAY PRECISION

Three samples were assayed in 20 different tests in the span of 20 days. The results are tabulated below.

Sample	Mean ( $\mu$ g/mL)	SD ( $\mu$ g/mL)	CV %
1	6.16	0.52	8.4
2	12.07	0.81	6.7
3	38.39	2.55	6.6



### RECOVERY

Three patient serum samples were spiked by adding defined amounts of adiponectin to samples that were initially diluted 1:1000 in working dilution buffer. The results are tabulated below.

Sample	Obs. Result	Exp. Result	Recovery %
1 - Unspiked	3.65	-	-
+ 5 µg/mL	8.74	8.65	101
+ 10 µg/mL	13.65	13.65	100
+ 20 µg/mL	23.58	23.65	99.7
2 - Unspiked	8.48	-	-
+ 5 µg/mL	13.25	13.48	98.3
+ 10 µg/mL	18.18	18.48	98.4
+ 20 µg/mL	25.88	28.48	90.9
3 - Unspiked	12.65	-	-
+ 5 µg/mL	16.28	17.65	92.2
+ 10 µg/mL	20.57	22.65	90.8
+ 20 µg/mL	28.19	32.65	86.3

### LINEARITY

Three patient serum samples that were initially diluted 1:1000 were serially diluted further with working dilution buffer. The results are tabulated below in µg/mL.

Sample	Obs. Result	Exp. Result	Recovery %
1	8.11	-	-
1:2	3.9	4.06	96.1
1:4	1.96	2.03	96.6
1:8	0.92	1.01	91.1
2	11.23	-	-
1:2	5.45	5.62	97.0
1:4	2.62	2.81	93.2
1:8	1.27	1.40	90.7
3	36.25	-	-
1:2	15.88	18.13	87.6
1:4	7.53	9.06	83.1
1:8	3.73	4.53	82.3

### COMPARATIVE STUDIES

The Eagle Biosciences Adiponectin ELISA kit (y) was compared with a leading competitor ELISA kit (x). The comparison of 40 serum samples yielded the following linear regression results:

$$y = 0.85x + 1.01, r = 0.98$$

### REFERENCE VALUES

As for all clinical assays each laboratory should collect data and establish their own range of reference values.

Group	N	Mean (µg/mL)	Range (ng/mL)
BMI <25	50	9.7	3.4-19.5
BMI 25-30	50	7.1	2.6-13.7
BMI >30	50	4.5	1.8-9.4



## REFERENCES

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3. Sekiyama K, et al. Circulating Proinflammatory Cytokines (IL-1 $\beta$ , TNF- $\alpha$ , and IL-6) and IL-1 Receptor Antagonist (IL-1Ra) in Fulminant Hepatic Failure and Acute Hepatitis. *Clin Exp Immunol*. 1994; 98(1):71–7.
4. Hara T, et al. Decreased Plasma Adiponectin Levels in Young Obese Males. *J Atheroscler Thromb*. 2003; 10(4):234–8.
5. Hanley AJG, et al. Adiponectin in a Native Canadian Population Experiencing Rapid Epidemiological Transition. *Diabetes Care*. 2003; 26(12):3219–25.
6. Blüher M, et al. Total and High-Molecular Weight Adiponectin in Relation to Metabolic Variables at Baseline and in Response to an Exercise Treatment Program: Comparative Evaluation of Three Essays. *Diabetes Care*. 2007; 30(2):280–5.

## Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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*For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at [info@eaglebio.com](mailto:info@eaglebio.com) or at 866-411-8023.*