Catalog #80570

V.7M

Rat Adiponectin ELISA Kit Instructions

For the quantitative determination of adiponectin in rat serum and plasma

Catalog #80570
96 Assays

For research use only. Not for use in diagnostic procedures.

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TABLE OF CONTENTS

A. Intended Use

The Rat Adiponectin ELISA kit is for the quantitative determination of adiponectin in rat serum and plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Adiponectin is a protein mainly synthesized by adipocytes, but also muscle cells and hepatocytes. Adiponectin is involved in regulation of energy and fat metabolism. Its concentration in circulation is thought to be correlated to the risk of atherosclerosis and the degree of insulin resistance.

Based on the high incidence of these diseases, adiponectin is the object of intensive research regarding the underlying biological mechanisms and regarding its value as biomarker. As a result, the accurate measurement of adiponectin in experimental animals is becoming increasingly important.

C. Principle of the Assay

The Rat Adiponectin ELISA kit is an ELISA sandwich assay for mouse adiponectin. The adiponectin in the mouse samples binds to the first antibody coated on the microtiter plate. In the following step, the second specific antibody binds in turn to the immobilized adiponectin. The second antibody is biotinylated and forms a complex with a streptavidin-peroxidase-enzyme conjugate. In the closing substrate reaction, the adiponectin levels of the samples can be measured by color intensity.

D. Kit Storage

1. Upon receipt of the Rat Adiponectin ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
STD1-6	Standards	1 x 6 vials
DIL	Dilution Buffer	1 x 125 mL
CON1-2	Controls	1 x 2 vials
AB CONJ	Antibody-POD Conjugate	1 x 12 mL
WASH	Wash Buffer (20X Concentrate)	1 x 50 mL
SUB	Substrate Solution	1 x 12 mL
STOP	Stop Solution	1 x 12 mL
	Sealing tape for plate	2x, adhesive

E.2. Materials required but not provided

Micropipettes and disposable tips
Distilled water
Polypropylene microtubes
Volumetric flasks
Vortex mixer
Microplate shaker (350 rpm)
Microplate reader (capable of reading A_{450} and A_{630} values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (5 µL), pipetting should be done as carefully as possible. A high quality 10 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Rat serum and plasma samples collected with EDTA or Heparin anticoagulant can be used. Hemolytic samples should be avoided. The blood has to be allowed to clot and after complete clotting, serum is separated by centrifugation. Samples should be stored no more than 2 days at room temperate and no more than 2 years at -20°C.

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use.
2. Standards 1-6
   Standards are provided in lyophilized form with concentrations ranging from 0.25 ng/mL to 10 ng/mL. Dilute each standard with 1 mL of Dilution Buffer (marked “DIL”). After reconstitution, it is recommended that standards be allowed to sit for 15 mins at room temperature and then mixed thoroughly but gently with a Vortex mixer. Reconstituted standards are recommended to be stored frozen at -20°C if not used all at once. Standards should be not be repeatedly thawed (at most, once), so standards should be appropriately aliquoted in appropriate volumes prior to being frozen. Standards are provided in the following concentrations: 0.25, 0.75, 1.5, 3, 6.5, and 10 ng/mL.

3. Dilution Buffer
   Provided as ready to use.
4. Controls 1-2
   Controls are provided in lyophilized form with target value and ranges included on their labels. Dilute controls with 250 µL of Dilution Buffer.
5. Antibody-POD Conjugate
   Provided as ready to use.
6. Wash Buffer (20X Concentrated)
   The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water. Wash buffer is stable for 4 weeks after dilution, so dilute only as needed.
7. Substrate Solution
   Provided as ready to use.
8. Stop Solution
   Provided as ready to use.

I.2. Dilution of samples and controls

1. Samples and controls need to be diluted with Dilution Buffer for use with the assay. Please note that this section only applies to samples and controls, not standards. A sample dilution of 1:1,500 is generally suitable and should be performed as follows:

   a. Dilute 1:50 by mixing 5 µL of sample or control with 245 µL of Dilution Buffer.
   b. Dilute another 1:30 by mixing 5 µL of prior mixture with 145 µL of Dilution Buffer. Sample (or control) is accordingly diluted 1:1,500.

   Since adiponectin levels can vary significantly, dilution ratio may need to be adjusted as appropriate.

I.3. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 100 µL of diluted sample or 100 µL of standard or 100 µL of diluted control.
   Note: *A blank using 100 µL of Dilution Buffer is recommended.*
2. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at 350 rpm).
3. Aspirate well contents and wash three times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
4. Add 100 µL of the Antibody-POD Conjugate in each well.
5. Cover the wells with sealing tape and incubate the plate for 1 hour at room temperature (shake at 350 rpm).
6. Aspirate well contents and wash three times using 300 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 µL of Substrate Solution in each well.
8. Incubate the plate for 30 mins in dark room at room temperature..
9. Stop the reaction by adding 100 µL of Stop Solution.
10. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.4. Determining the adiponectin concentration

1. Using computer software, construct the adiponectin calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding adiponectin concentration on the X axis. A higher-grade polynomial, or four parametric logistic (4-PL) curve fit or non-linear regression are suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. Rat adiponectin concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. For diluted samples and controls, the values obtained must be multiplied by the dilution factor (ie. 1,500) to obtain the final adiponectin concentration. The adiponectin concentration is expressed in ng/mL.
   Note: *Samples with high rat adiponectin concentrations (ie. fall above the range of the assay) should be further diluted with the dilution buffer and rerun.*

J. Performance characteristics

J.1. Assay range

The Rat Adiponectin ELISA Kit has an assay range from 0.25 – 10 ng/mL.

J.2. Precision

The assay has a within-run and total precision of CV < 10%.

Warranty

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