Rat Adiponectin ELISA Assay Kit

(Catalog #K4903-100; Store kit at +4°C)

I. Description:

Adipose tissue secretes a number of biologically active soluble factors (collectively named adipocytokines) that regulate glucose and fatty acid metabolism. Measurement of serum adiponectin levels gives us important information on the role of adiponectin in regulation of glucose and/or lipid metabolism. This Rat Adiponectin ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of adiponectin in rat serum, plasma or various tissue or cell culture supernatants. In the assay, polyclonal antibody specific for rat adiponectin has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and any adiponectin present is bound by immobilized antibody. The bound adiponectin is then captured by anti-rat adiponectin monoclonal antibody. With adding HRP conjugated anti-mouse IgG and HRP substrate, the colors developed in proportion to the bound adiponectin, can be easily measured by Elisa plate reader.

II. Kit Components:

Components	K4903-100	Part No.
Plate coated with rat adiponectin ab	6x16-well strips	K4903-100-1
Wash Buffer (10x)	2x30 ml	K4903-100-2
ELISA Buffer (10x)	2x30 ml	K4903-100-3
Detection Antibody (DET)	20 µl	K4903-100-4
HRP conjugated anti-mouse IgG (100X)	150 µl	K4903-100-5
Rat adiponectin, Standard (lyophilized)	48 ng	K4903-100-6
TMB Substrate	12 ml	K4903-100-7
Stop Solution	12 ml	K4903-100-8
Plate sealers	2	K4903-100-9

III. Storage Conditions:

Reagents must be stored at 2-8°C when not in use. The reagents must be brought up to room temperature before use. Do not expose the reagents to temperature above 25°C.

IV. Assay Procedure

A. Preparation of Reagents

Note: Prepare just the appropriate amount of the buffers necessary for the assay.

1. Allow all samples and kit components to equilibrate to room temperature (20-25°C).
2. Plan the plate configuration and create a plate map. Calculate the amount of working reagents to use (See table below).

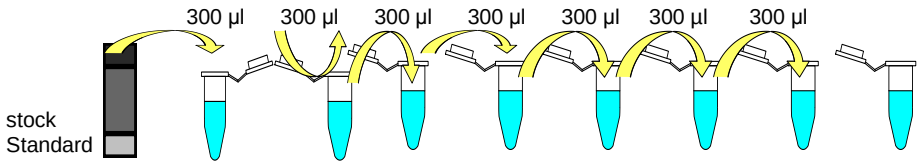
   It is recommended that standards and samples be run in duplicate.
3. Prepare **1X Wash Buffer:** Dilute 10X Wash buffer to 1X with deionized water.
4. Prepare **1X ELISA Buffer**. Diluent to 1X with deionized water.
5. **Detection Antibody (DET)** has to be diluted to 1:1'000 in ELISA Buffer 1X. *NOTE: The diluted Detection Antibody is not stable and cannot be stored!*
6. **HRP conjugated anti-mouse IgG (100X):** has to be diluted to the working concentration by adding 100 µl in 10 ml of ELISA Buffer 1X (1:100).

NOTE: *The diluted HRP is used within one hour of preparation.*

7. Prepare working aliquots of the Standard as follows:

   When opening the lyophilized satndard, remove cap gently as the lyophilizate may have become dislodged during shipping. Add 1 ml of deionized water to the Standard vial to make a stock concentration of 48 ng/ml. Mix well. Allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions. A recommended dilution scheme is as follows:

1) Label 8 microcentrifuge tubes #0 – 7 and add 300 µl 1X ELISA Buffer to each microcentrifuge tube.
2) Add 300 µl of the stock Standard solution to tube #7 and vortex. This is Standard tube #7 with a concentration of 24 ng/ml
3) Standards #6 to #1 are then prepared by performing a 1:2 dilution of the preceding standard. Do not add any standard to the tube #0



Tube #	stock	7	6	5	4	3	2	1	0
Conc.(ng/ml)	48	24	12	6	3	1.5	0.75	0.375	0
Initial vol.(µl)	-	300	300	300	300	300	300	300	300

B. Sample dilution

Serum : Use a serum separator tube. Let samples clot at room temperature for 30 minutes before
centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at
≤ -20°C for later use. Avoid repeated freeze/thaw cycles.
Plasma : Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma
sample in aliquot at ≤ -20°C for later use. Avoid repeated freeze/ thaw cycles.
Serum, Plasma or **Cell Culture Supernatant** have to be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use.
NOTE: *As a starting point, 1/1,000 dilution of serum or plasma are recommended! If sample values fall outside the detection range of the assay, a lower or higher dilution may be required!*

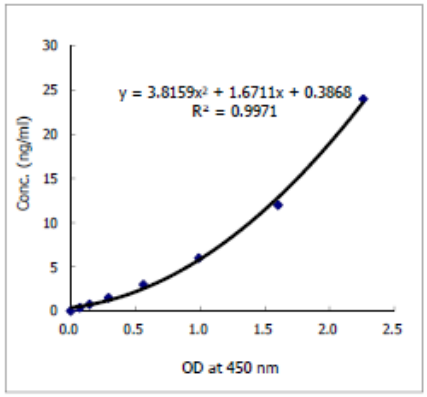
C. Experiment procedure

1. Remove the appropriate number of microwell strips from the sealed foil pouch.
2. Pipette 100 µl of standards 0 to 7, and diluted serum sample into the antibody-coated plate in duplicate to the wells. Use a new pipette tip for each standard or sample.
3. Incubate at 37°C for 1 hr.

4. Remove the solution and wash 3 times with 300 µl of 1X Wash buffer to each well.
5. Add 100 µl detection Antibody to each well.
6. Incubate at 37°C for 1 hr.
7. Remove the solution and wash 3 times with 300 µl of 1X Wash buffer to each well.
8. Add 100 µl diluted HRP conjugated anti-mouse IgG to each well.
9. Incubate at 37°C for 1 hr.
10. Remove the solution and wash 5 times with 300 µl of 1X Wash buffer to each well.
11. Add 100 µl of the TMB Substrate Solution to each well.
12. Incubate at room temperature for 20 min. Protect from light.
13. Stop reaction by adding 100 µl Stop Solution to each well.
14. Read absorbance at 450 nm within 30 min..
15. Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 ng/ml point).
16. Construct a standard curve by plotting the known concentrations (Y) of standard versus the absorbances (X) of standard. A measurable range is typically shown between 0.375 ng/ml and 24 ng/ml.
17. Calculate the adiponectin concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation.
18. The adiponectin concentrations calculated must be multiplied by dilution factor to obtain the concentrations of the undiluted samples.

Typical data

The following data are obtained using the different concentrations of standard as described in this protocol:



Standard rAdiponectin (ng/ml)	Optical Density (mean)
24	2.260
12	1.602
6	0.989
3	0.561
1.5	0.292
0.75	0.146
0.375	0.071
0	0

Figure: Standard curve

IV. Performance Characteristics:

A. **Sensitivity:** The limit of detection: 50 pg/ml. ***NOTE:*** *The Limit of detection was measured by adding two standard deviations to the mean value of 50 zero standard.*

B. **Assay range:** 0.375 ng/ml – 24 ng/ml

C. **Specificity:** This ELISA is specific for the measurement of natural and recombinant rat adiponectin. It does not cross-react with mouse adiponectin, human adiponectin, rat Nampt, rat resistin, rat RELM-α, rat leptin, human TNF-α.

d. Recovery: The average recovery of adiponectin is 87-105%.

Expected values: Adiponectin levels range in plasma and serum from **3 to >7 µg/ml** (from normal rats).

RELATED PRODUCTS:

- Recombinant Adiponectin Proteins, Antibodies, and Elisa Kits
- Recombinant Resistein, Leptin, Visfatin Proteins, Antibodies, Elisa Kits
- Cholesterol and HDL/LDL Quantification Kits
- Glucose, Lactate, Uric Acid, Ascorbic Acid and Other Metabolism Assay Kits
- CETP and PLTP Assay and Drug Discovery Kits
- Apoptosis Assay Kits and Reagents
- Cell Proliferation and Cell Death Assays
- Cellular Fractionation Kits
- Glutathione, Nitric Oxide and Other Stress Related Assays
- cAMP/cGMP, Kinase, Secretase and Other Cell signaling Assays kits
- HDAC and HAT Assay Kits and Drug Discovery
- DNA Damage, SOD Quantification Kits
- siRNA Expression Vectors
- Recombinant Growth Factors and Cytokines
- Polyclonal and Monoclonal antibodies

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