Human Adiponectin ELISA Assay Kit

(Catalog #K4901-100; 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 human Adiponectin ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of adiponectin in human serum, plasma or various tissue or cell culture supernatants. In the assay, monoclonal antibody specific for human adiponectin has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells and adiponectin present is bound by immobilized antibody. The bound adiponectin is then captured by anti-human adiponectin polyclonal antibody. After removal of excess polyclonal antibody, HRP conjugated anti-rabbit IgG (HRP) is added. Following a final washing, peroxidase activity is quantified using TMB substrate. The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of adiponectin in the samples.

II. Kit Components:

Components	K4901-100	Part No.
Plate coated with antibody	6 x 16-well strips	K4901-100-1
Wash concentrate (10X)	2 X 30 ml	K4901-100-2
ELISA Buffer (10X)	2 X 30 ml	K4901-100-3
Detection Antibody	30 µl	K4901-100-4
HRP 100X (HRP Conjugated anti-rabbit IgG)	150 µl	K4901-100-5
recombinant human adiponectin Standard (lyophilized)	64 ng	K4901-100-6
TMB Substrate Solution	12 ml	K4901-100-7
Stop Solution	12 ml	K4901-100-8
Plate Sealer	2	K4901-100-9
Silica Gel Minibags	2	K4901-100-10

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. Diluted wash solution may be stored at room temperature for up to one month.

IV. Assay Procedure

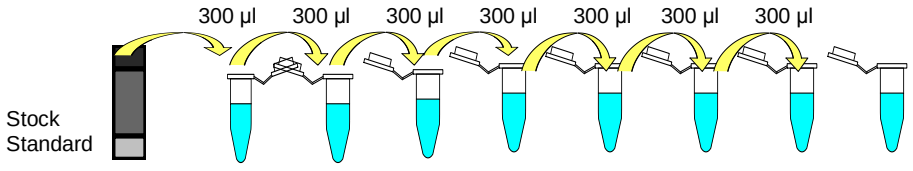
A. Preparation of Reagents

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 Solution:** Dilute 10X Wash Concentrate to 1X with deionized water. The diluted 1X Wash Solution is stable for one month at room temprature
4. Prepare **1X ELISA Buffer.** ELISA Buffer 10X has to be diluted with deionized water 1:10 before use.
5. **Detection Antibody (DET)** has to be diluted to 1:1000 in ELISA Buffer 1X. (NOTE: The diluted Detection Antibody is not stable and cannot be stored.)
6. HRP 100X (HRP Conjugated anti-rabbit IgG) has to be diluted to the working concentration by adding 100 µl in 10 ml of ELISA Buffer 1X (1:100).
7. Prepare working aliquots of the Standard as follows:

   Briefly centrifuge the lyophilzed Standard vial. When opening, remove cap gently as the lyophilizate may have become dislodged during shipping. Add 1 ml of deionized water the Standard vial to make a stock concentration of 64 ng/ml. Mix well. A recommended dilution scheme is as follows:

   1) Label 8 microcentrifuge tubes #0 – 7 and add 300 µl Diluent 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 32 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
   4) Suggested standard points: 32, 16, 8, 4, 2, 1, 0.5, 0 ng/ml.



Tube #	stock	7	6	5	4	3	2	1	0
Conc.(ng/ml)	64	32	16	8	4	2	1	0.5	0
Initial vol.(µl)	-	300	300	300	300	300	300	300	300

B. Sample Collection, Storage and Dilution

Note: As a starting point, 1/2,000 dilution of serum or plasma and 1/10 dilution of urine are recommended! If sample values fall outside the detection range of the assay, a lower or higher dilution may be required.

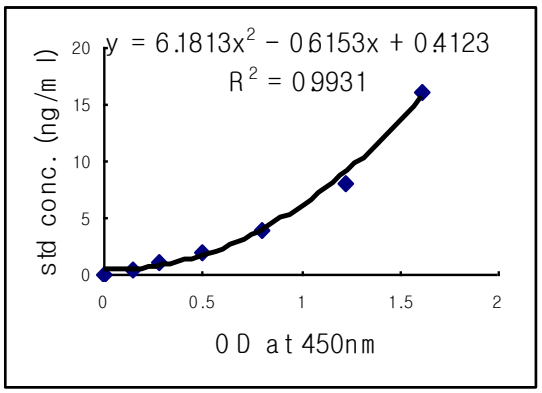
- **Serum**: Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at ≤ -20°C for later use. Avoid repeated freeze/thaw cycles.
- **Plasma**: Collect plasma using heparin, 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.
- **Urine**: Aseptically collect the urine of the day, voided directly into a sterile container. Assay immediately or aliquot and store at ≤ -20oC. Avoid repeated freeze/thaw cycles. Serum, Plasma, Urine or Cell Culture Supernatant have to be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use.

C. Experiment procedure

1. Remove the appropriate number of microwell strips from the sealed foil pouch. (Remaining strips when opened can be stored at 4°C for up to 1 month.)
2. Add 100 µl of the different standards into the appropriate wells in duplicate. At the same time, add 100 µl of diluted serum, plasma, urine or cell culture supernatant samples in

duplicate to the wells3.

3. Cover the plate with plate sealer and incubate for 1 hour at 37°C
4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
5. Add 100 µl Detection Antibody to each well.
6. Cover the plate with plate sealer and incubate for 1 hour at 37°C.
7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
8. Add 100 µl 1X HRP to each well.
9. Incubate at 37°C for 1 hr.
10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.
11. Add 100 µl of the TMB Substrate Solution to each well.
12. Incubate at room temperature for 20 min. Protect in the dark.
13. Stop the reaction by adding 100 µl of Stop Solution (STOP). Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution (STOP) is added.
14. Read absorbance at 450 nm within 30 minutes.
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. Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding adiponectin concentration (ng/ml) on the vertical (Y) axis (see 10. TYPICAL DATA).
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. If the test samples were diluted, multiply the interpolated values by the dilution factor to calculate the concentration of human adiponectin in the samples.



V. Performance Characteristics:

a. **Sensitivity:** The limit of detection: 100 pg/ml.

b. **Assay Range:** 0.5 ng/ml – 32 ng/ml

c. **Specificity:** This ELISA is specific for the measurement of natural and recombinant human adiponectin. No cross-reaction with mouse and rat adiponectin.

d. **Recovery:** The average recovery range for human serum samples is 87 - 102%. The average recovery range for human urine samples is 97 - 105%.

RELATED PRODUCTS:

- Recombinant Adiponectin Proteins, Antibodies, and Elisa Kits
- Recombinant Resistin, 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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