Product Information

Adiponectin (human) Elisa Assay Kit

I. Kit Contents:

Components	K2192-100 100 assays	Part Number
Plate coated with antibody	12 stripsx8 wells	K2192-C-1
Wash concentrate (10x)	50 ml	K2192-C-2
Diluent 5X	50 ml	K2192-C-3
Secondary Antibody	12 ml	K2192-C-4
Detector 100X	150 µl	K2192-C-5
recombinant human adiponectin Standard (lyophilized)	64 ng	K2192-C-6
Human adiponectin QC sample (lyophilized)	1 Vial	K2192-C-7
Substrate I	6 ml	K2192-C-8
Substrate II	6 ml	K2192-C-9
Stop Solution	12 ml	K2192-C-10

II. Introduction:

Adipose tissue secretes various biologically active soluble factors that regulates metabolism of glucose and fatty acid. The Adiponectin (human) Elisa Assay Kit is an enzyme-linked immunosorbent assay for quantitative analysis of andiponectin in human serum, plasma and tissue/cell culture supernatants etc. In this assay, human adiponectin specific monoclonal antibody has been pre-coated onto 96 well microplate. Standards and samples are pipetted into the wells. The adiponectin present is than attached to the immobilized antibody. The bound adiponectin is then captured by anti-human adiponectin polyclonal antibody. With HRP conjugated anti-rabbit IgG and a HRP substrate, the colors generated is proportion to the bound adiponectin, can be easily measured by Elisa plate reader.

III. Storage Conditions:

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

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 Diluent. Dilute 5X Diluent to 1X with deionized water.

5. Prepare 1X Detector. Dilute 100X Detector to 1X with 1X Diluent. Use the 1X Detector within one hour of preparation.

6. Prepare Substrate Solution freshly by adding one part Substrate I to one part Substrate II. Freshly prepare just before use.

The amount of working reagents needed for 1 well

Working reagents	Total volume needed	Stock solution added	Dilution solution added	Note

1X Wash Solution	2.8 ml	0.56 ml of 5X Wash Concentrate	2.24 ml of ddH_2O	Stable for 1 month at RT
1X Diluent	2.5 ml	0.5 ml of 5X Diluent	2.0 ml of ddH_2O	in the case of 10 µl sample; Including standard dilution
1X Detector	110 µl	1.1 µl of 100X Detector	108.9 µl of 1X Diluent	Use within 1 hr.
Substrate Solution	110 µl	55 µl of Substrate I	55 µl of Substrate II	Freshly prepared just before use

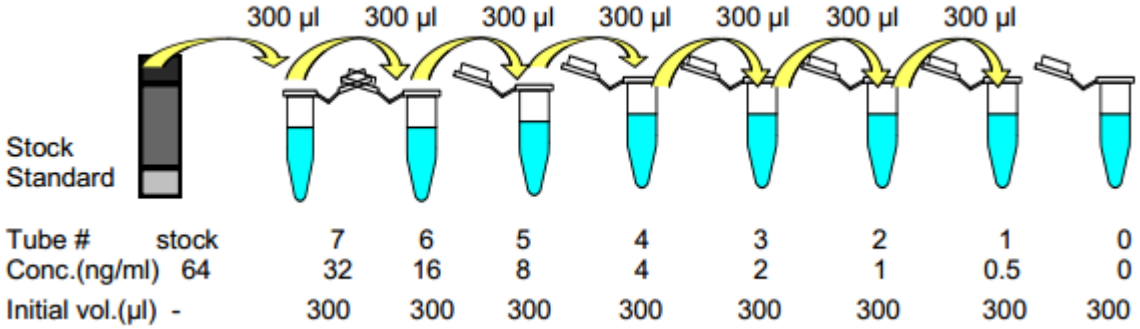
7. Prepare working aliquots of the Standard as follows:

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 200 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



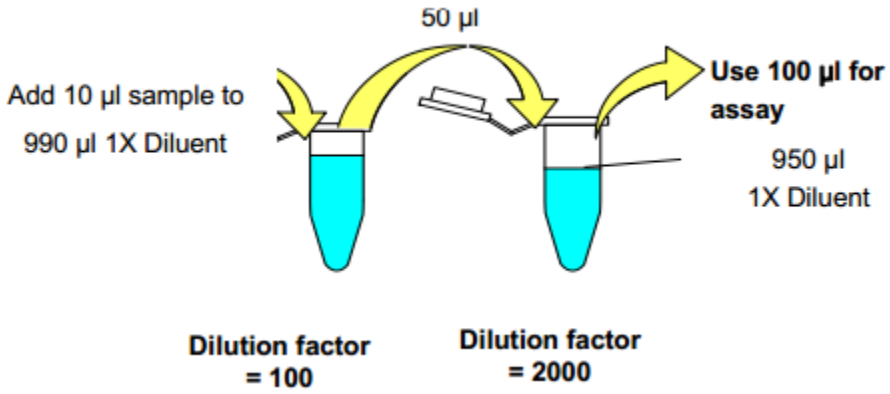
8. Reconstitute QC sample in 1 ml of deionized water. Reconstituted QC sample concentration: 7 - 11 µg/ml.

B. Sample dilution

Step 1. Dilute samples 1:100 with 1X Diluent (for example, 10 µl sample plus 990 µl 1X Diluent, final 1:100)

Step 2. Dilute the samples (from step 1) 1:20 with 1X Diluent (for example, 50 µl step 1 sample plus 950 µl 1X Diluent, final 1:2000)

If samples fall the outside range of assay, a lower or higher dilution may be required. Step3. Use 100 µl of the final sample for ELISA.



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, the reconstituted QC sample and pre-treated plasma

sample into the antibody-coated plate according to the plate configuration. Use a new pipette tip for each standard or sample.

3. Incubate at 37℃ for 1 hr.

4. Remove the solution and wash 3 times with 250 µl of 1X Wash Solution to each well.

5. Add 100 µl Secondary Antibody to each well.

6. Incubate at 37℃ for 1 hour.

7. Remove the solution and wash 3 times with 250 µl of 1X Wash Solution to each well.

8. Add 100 µl 1X Detector to each well.

9. Incubate at 37℃ for 1 hr.

10. Remove the solution and wash 5 times with 250 µl of 1X Wash Solution to each well.

11. Using the multi-channel pipette, add 100 µl of the Substrate Solution to each well.

12. Incubate at room temperature for 10-20 min. Protect from light.

13. Using the multi-channel pipette, add 100 µl Stop Solution to each well.

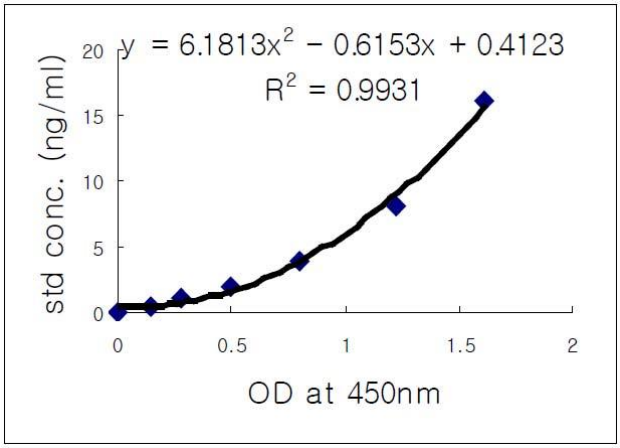
14. Read absorbance at 450 nm.

15. Subtract the absorbance of the blank from the readings for each standard and sample.

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 0.5 ng/ml and 32 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 (Dilution factor of lyophilized QC sample is 2000)



V. Performance Characteristics:

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

b. Specificity: No cross-reaction with mouse and rat adiponectin.

c. Recovery: The average recovery of adiponectin is 90 - 105%.

For research use only! Not to be used in humans.

Our promise

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For more details, please visit http://www.apexbt.com/ or contact our technical team.

Tel: +1-(832)696-8203
Fax: +1-832-641-3177
Email: sales@apexbt.com