Product Information

Adiponectin (mouse) Elisa Assay Kit

I. Kit Contents:

Components	K2193-100 100 assays	Part Number
Adiponectin antibody-coated plate	6 x 16 wells	K2193-C-1
Wash concentrate (10X)	2 x 30 ml	K2193-C-2
Diluent (10X)	2 x 30 ml	K2193-C-3
Secondary Antibody (200X)	60 µl	K2193-C-4
Detector (100X)	150 µl	K2193-C-5
Mouse adiponectin Standard (lyophilized)	16 ng	K2193-C-6
TMB	12 ml	K2193-C-7
Stop Solution	12 ml	K2193-C-8
Plate sealers	2 ml	K2193-C-9

II. Introduction:

Adipose tissue secretes various biologically active soluble factors that regulates metabolism of glucose and fatty acid. The Adiponectin (Mouse) Elisa Assay Kit is an enzyme-linked immunosorbent assay for quantitative analysis of andiponectin in mouse serum, plasma and tissue/cell culture supernatants etc. In this assay, mouse 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. 1X Wash Solution: Dilute 10X Wash Concentrate to 1X with deionized water. The diluted 1X Wash Solution is stable for one month at RT.

4. 1X Diluent. Dilute 10X Diluent to 1X with deionized water.

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

6. 1X Secondary Antibody: Dilute 200X to 1X with 1X Diluent. Use within 1 hour.

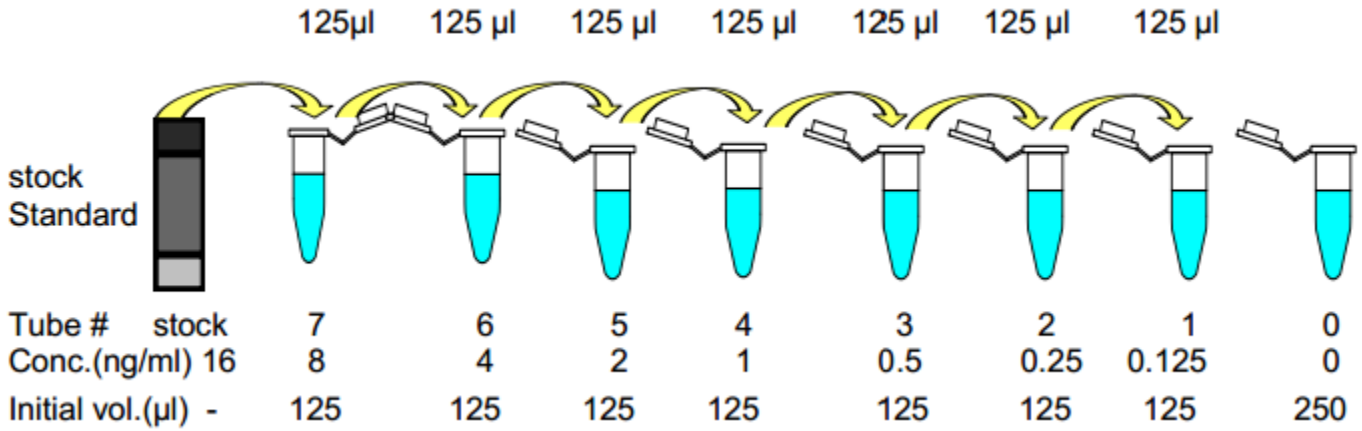
7. Prepare working aliquots of the Standard as follows:

Briefly centrifuge the lyophilized Standard vial. When opening, remove cap gently as the lyophilizate may have become dislodged during shipping. Add 1 ml of deionized water to make a stock concentration of 16 ng/ml. Mix well. Aliquot and store at - 20℃ for future use. A recommended dilution scheme is as follows:

1) Label 8 microcentrifuge tubes #0 – 7 and add 125 µl Diluent to each microcentrifuge tube.

2) Add 125 µl of the stock Standard solution to tube #7 and vortex. This is Standard tube #7 with a concentration of 8 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

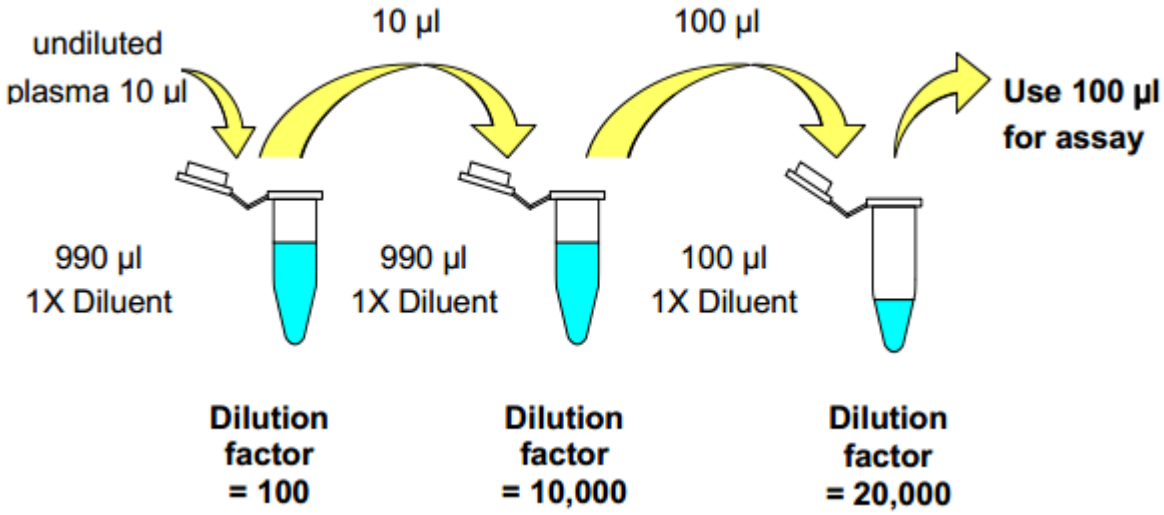


2) Sample dilution (1: 20,000 dilution recommended for plasma/serum)

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

Step 2. Dilute the samples from step 1, 1:100 with 1X Diluent (for example, 10 µl step 1 sample plus 990 µl 1X Diluent, final 1:10,000)

Step 3. Dilute the step 2 sample solution 1:2 with 1X diluent (example, 100 µl of 2 step plus 100 µl 1X Diluent, final dilution factor = 20,000. If samples fall the outside range of assay, a lower or higher dilution may be required..



1. Remove the appropriate number of microwell strips from the sealed foil pouch.

2. Pipette 100 µl of Standard 0 to 7 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. Cover the plate with plate sealer and incubate at 37℃ for 1 hr.

4. Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.

5. Add 100 µl 1X Secondary Antibody to each well.

6. Cover the plate with plate sealer and incubate at 37℃ for 1 hr.

7. Remove the solution and wash 3 times with 300 µl of 1X Wash Solution to each well.

8. Add 100 µl 1X Detector to each well

9. Cover the plate with plate sealer and incubate at 37℃ for 1 hr.

10. Remove the solution and wash 5 times with 300 µl of 1X Wash Solution to each well.

11. Add 100 µl of the TMB Solution to each well.

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

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

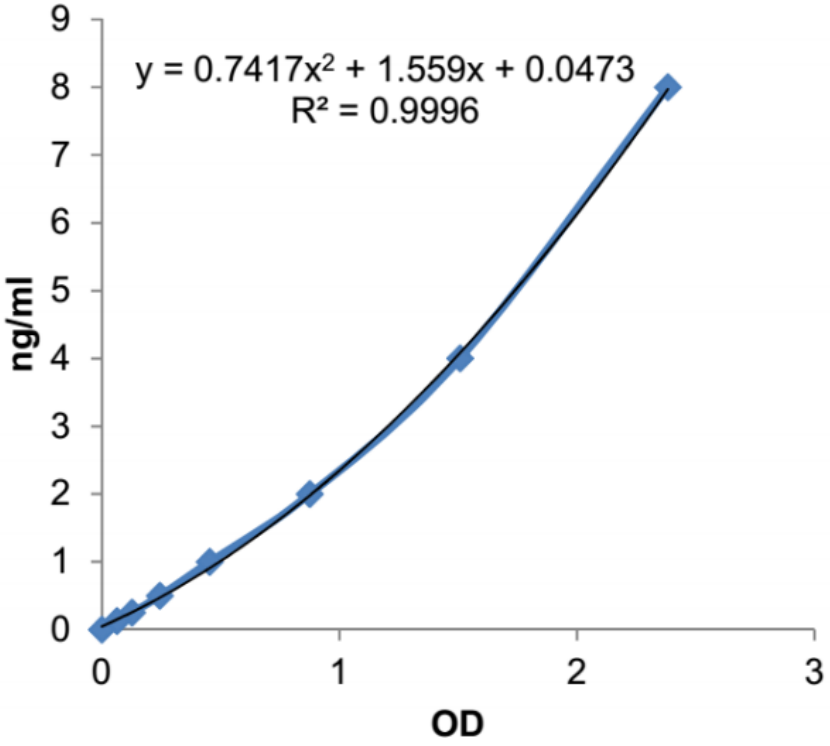
14. Read absorbance at 450 nm.

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

16. Construct the standard curve by plotting the known concentration (X) of standard versus the absorbance (Y) of standard.A typical linear range is shown between 0.125 ng/ml and 2 ng/ml.

17. Calculate the adiponectin concentrations of samples by interpolation of the quadratic regression curve formula.

18. The adiponectin concentrations calculated must be multiplied by dilution factor to obtain the concentrations of the undiluted sample



V. Performance Characteristics:

a. Sensitivity: The limit of detection: 50 pg/ml.

b. Specificity: No cross-reaction with human and mouse sera.

c. Recovery: The average recovery of adiponectin is 90 - 100%.

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