Mouse Adiponectin ELISA Assay Kit

(Catalog #K4902-100; 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 Mouse Adiponectin ELISA Kit is an enzyme-linked immunosorbent assay (ELISA) for quantitative determination of adiponectin in mouse serum, plasma or various tissue or cell culture supernatants. In the assay, monoclonal antibody specific for mouse 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-mouse adiponectin polyclonal antibody. With HRP conjugated anti-rabbit IgG and a HRP substrate, the colors developed in proportion to the bound adiponectin, can be easily measured by Elisa plate reader.

II. Kit Components:

1) Antibody coated 96-well plate
2) 5X Wash concentrate, 100 ml
3) 5X Diluent, 50 ml
4) Secondary antibody, 12 ml
5) 100X Detector, 150 µl
6) Standard, recombinant mouse adiponectin (16 ng), 1 vial, lyophilized
7) QC sample = positive control having 16 - 22 µg/ml of mouse serum adiponectin
8) Substrate I, 6 ml
9) Substrate II, 6 ml
10) Stop solution, 12 ml

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 5X 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.

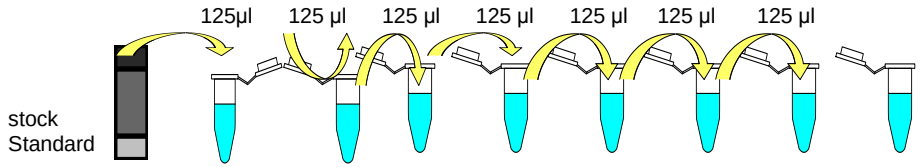
The amount of working reagents needed for 1 well

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 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 repared 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 48 ng/ml. Mix well. 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 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)	16	8	4	2	1	0.5	0.25	0.125	0
Initial vol.(µl)	-	125	125	125	125	125	125	125	250

8. Reconstitute QC sample in 1 ml of deionized water.

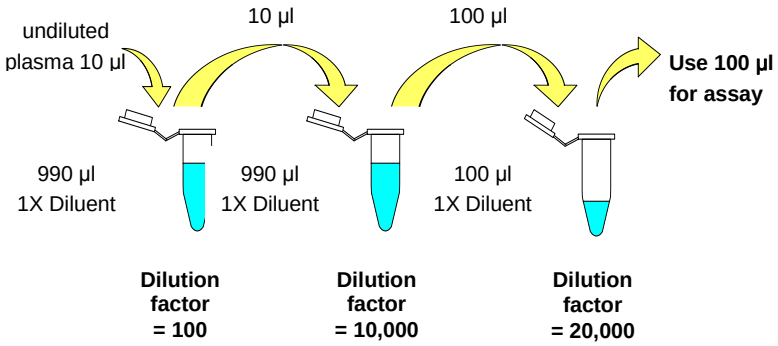
2) Sample dilution

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)

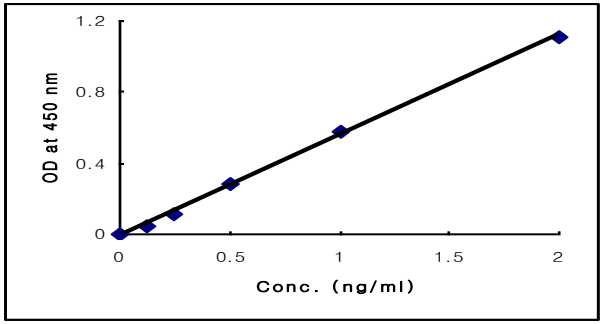
Step 3. Dilute the step 2 1:2 with 1Xdiluent (example, 100 µl of 2 step plus 100 µl 1X Diluent, dilutionfactor = 20,000

* If samples fall the outside range of 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 Standard 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°C 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°C for 1 hr.
7. Remove the solution and wash 3 times with 250 µl of 1X Wash Solution to each well.
8. Add 100µl 1X Detector to each well
9. Incubate at 37°C for 1 hour.
10. Remove the solution and wash 5 times with 250 µl of 1X Wash Solution to each well.
11. Add 100 µl of the Substrate Solution to each well.
12. Incubate at room temperature for 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 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 regression curve formula.
18. The adiponectin concentrations calculated must be multiplied by dilution factor [see 2) **Sample dilution**] to obtainthe concentrations of the undiluted sample (Dilution factor of lyophilized QC sample is 1000)



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 aversage recovery of adiponectin is 90-100%.

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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