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 (HRP Conjugate) and a HRP substrate, the colors developed in proportion to the bound adiponectin, can be easily measured by Elisa plate reader.

II. Kit Components:

Components	K4902-100	Part No.
Adiponectin antibody-coated plate	6 x 16 wells	K4902-100-1
Wash concentrate (10X)	2 x 30 ml	K4902-100-2
ELISA Buffer (10X)	2 x 30 ml	K4902-100-3
Detection Antibody	60 µl	K4902-100-4
HRP Conjugate (100X)	150 µl	K4902-100-5
Standard (lyophilized)	16 ng	K4902-100-6
TMB	12 ml	K4902-100-7
Stop Solution	12 ml	K4902-100-8
Plate sealers	2	K4902-100-9

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

IV. Assay Procedure

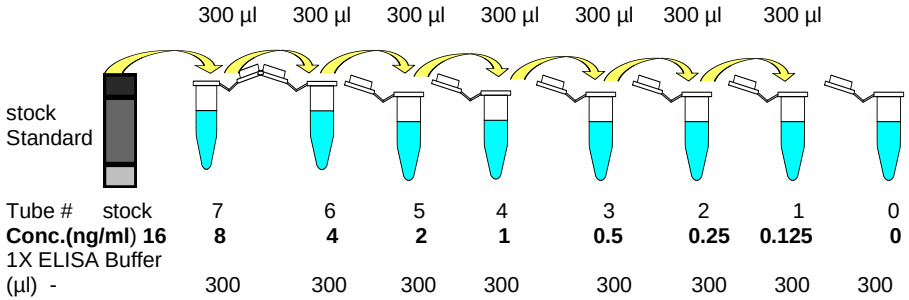
A. Preparation of Reagents

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. **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 ELISA Buffer:**. Dilute 10X ELISA Buffer to 1X with deionized water.
5. **HRP Conjugate**. Dilute 100X HRP Conjugate to 1X with 1X ELISA Buffer. Use the 1X HRP Conjugate within one hour of preparation.
6. **1X Detection Antibody**: Dilute 1:200 in 1X ELISA Buffer. 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˚ C for future use. 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 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 Preperation:

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.

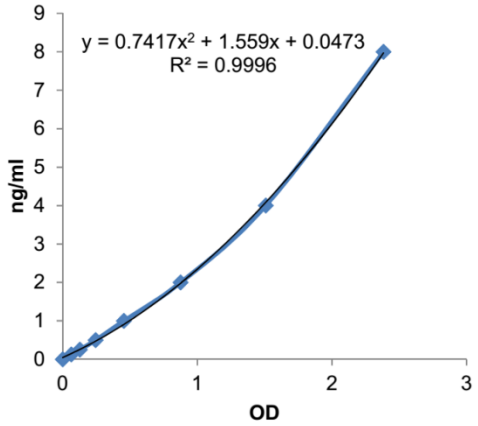
NOTE: As a starting point, 1/20,000 dilution of serum or plasma are recommended! If samples values fall outside the detection range of the assay, a lower or higher dilution may be required!

C. Experiment procedure

1. Remove the appropriate number of microwell strips from the sealed foil pouch.
2. Pipette 100 µl of **Standard** 0 to 7 and 100 µl of diluted **Samples** 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˚ C 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 Detection Antibody** to each well.
6. Cover the plate with plate sealer and incubate at 37˚ C 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 HRP Conjugate** to each well
9. Cover the plate with plate sealer and incubate at 37˚ C 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.

D. Calculation:

- 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.
- Calculate the adiponectin concentrations of samples by interpolation of the quadratic regression curve formula.
- The adiponectin concentrations calculated must be multiplied by dilution factor to obtain the concentrations of the undiluted sample.



Standard mAdiponectin (ng/ml)	Optical Density (mean)
8	2.382
4	1.508
2	0.875
1	0.454
0.5	0.244
0.25	0.127
0.125	0.064
0	0

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%.

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
- Recombinant Growth Factors and Cytokines
- Polyclonal and monoclonal antibodies

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