Reagent for research purposes only

** Revised: June, 2006
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Mouse/Rat adiponectin ELISA kit
Instructions for use

This kit is intended for research purposes use only, not for diagnosis or its aid.

[Background of development and characteristics]

Mouse adiponectin is a molecule identified by the group of Lodish et al., Massachusetts Institute of Technology, in 1995 as an Acrp30 (adipocyte complement-related protein of 30kD) which is an analogue of complement component C1q induced during differentiation of mouse 3T3-L1 cell.[1)] An adipoQ isolated from adipose cell differentiated from mouse 3T3-F442A cell by the group of Spiegelman et al. is the same molecule as Acrp30.[2)] On the other hand, adipocytokine identified by the group of Matsuzawa et al., Department of Internal Medicine and Molecular Science, Osaka University, in 1996 as a genetic apM1[3, 4)] (adipose most abundant gene transcript) expressed specifically in human adipose tissues has 83% homology with Acrp30 or adipoQ in their amino acid sequences, which showing that they are the counterparts each other. In the recent study, it has been demonstrated that the Knock-out mouse in which adiponectin gene has been turned off is more likely to develop insulin resistance and arteriosclerotic lesion than wild-type mouse.[5)] The relationships between decrease in adiponectin level and metabolic syndrome like diabetes mellitus[6, 7)], and arteriosclerosis[6, 8)] in human have been reported.[9)]

This kit is based on the enzyme-linked immunosorbent assay (ELISA) developed in a cooperative study with Matsuzawa, et al., and a recombinant mouse adiponectin is used as the reference standard. This kit enables measurement of adiponectin in mouse and rat serum and adipocyte extract or culture supernatant specifically, accurately, and simply.

[Composition of the kit]

	Constituent reagent	Volume	Ingredient, etc.
1	Stock washing solution	40 mL/bottle	Buffer, etc.
2	Stock specimen diluent	50 mL/bottle	Buffer, etc.
3	Antibody plate	96 wells/plate	Anti-mouse adiponectin polyclonal antibody (rabbit), etc.
4	8.0 ng/mL reference standard	2 mL/tube	Recombinant mouse adiponectin, etc.
5	Biotin-labeled antibody solution	12 mL/bottle	Biotin-labeled anti-mouse adiponectin polyclonal antibody (rabbit), etc.
6	Enzyme-labeled Streptoavidin stock solution	0.1 mL/tube	Horseradish peroxidase-labeled Streptoavidin, etc.
7	Enzyme-labeled Streptoavidin diluent	15 mL/bottle	Buffer, etc.
8	Substrate solution A	7.5 mL/bottle	3,3',5,5'-tetramethylbendizine, etc.
9	Substrate solution B	7.5 mL/bottle	Hydrogen peroxide, etc.
10	Reaction stopper solution	15 mL/bottle	Sulfuric acid, etc.

Accessory: 6 plate seals

[Purpose of use]

Measurement of mouse and rat adiponectin in serum as well as in adipocyte extract or culture supernatant

[Principle of measurement]

This product is a kit for measurement of mouse and/or rat adiponectin using ELISA.

The principle of measurement is shown in Fig. 1. When a specimen or the standard solution diluted in advance is added to a plate immobilized with anti-mouse adiponectin polyclonal antibody (rabbit) (antibody plate) to allow reaction, adiponectin binds to the antibody plate (first reaction). The biotin-labeled anti-mouse adiponectin

polyclonal antibody (rabbit) (Biotin-labeled antibody solution) is allowed to react (second reaction), followed by reaction with the horseradish peroxidase-labeled streptoavidin (Enzyme-labeled streptavidin solution) (third reaction). A substrate solution is added to develop color (coloring reaction), and the concentration of adiponectin in the specimen is calculated from the absorbance measured at 450 nm and the absorbance of the standard solution measured simultaneously. Rat adiponectin concentration is calculated as the equivalent of mouse adiponectin concentration.

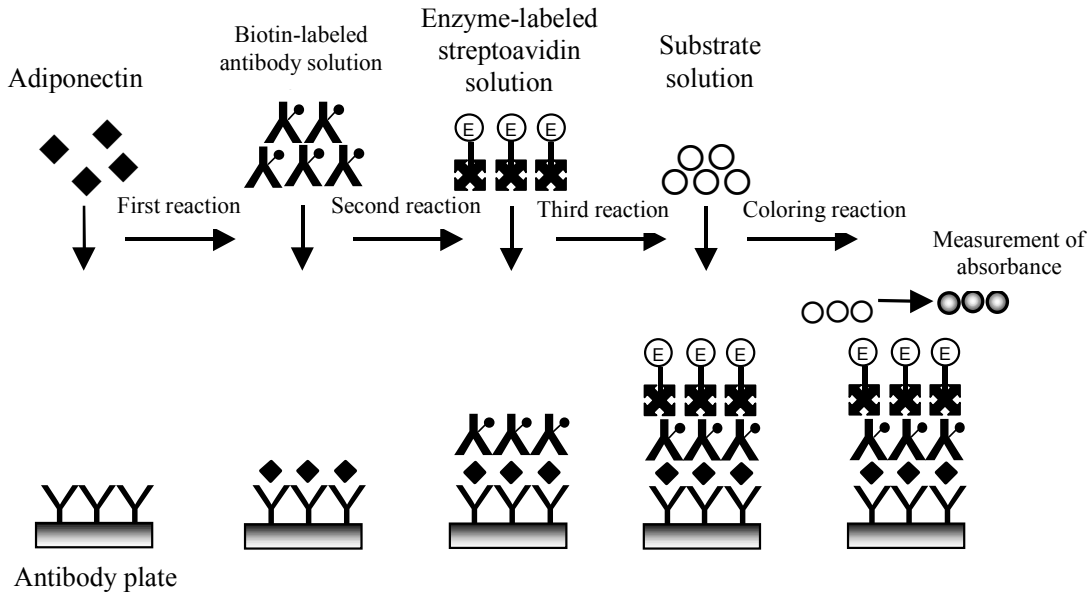

Fig. 1. Principle of Measurement

[Method of Measurement]

1. Apparatus, instruments, etc.

(1) Graduated cylinder
(2) Measuring pipette
(3) Micropipette and tip
(4) Plate washer
(5) Paper towel
(6) Plate reader (measurement wavelength: 450 nm)
(7) Microtube, etc. (sealable vial of 1.5 mL or more in volume)

2. Preparation and storage of reagents

(1) Washing solution

Mix the whole volume (40 mL) of the stock washing solution with 960 mL of purified water. If crystals have precipitated in the stock washing solution, dissolve them by warming before preparation. Store the solution at 2 – 8 ºC after preparation.

(2) Specimen diluent

Mix the whole volume (50 mL) of the stock specimen diluent with 200 mL of purified water. Store the solution at 2 – 8 ºC after preparation.

(3) Standard solution

Dilute the 8.0 ng/mL reference standard stepwise with the specimen diluent to obtain working standard solutions of 4.0, 2.0, 1.0, 0.50, and 0.25 ng/mL. Use the 8.0 ng/mL reference standard as the standard solution of 8.0 ng/mL and the specimen diluent as the standard solution of 0 ng/mL.

(4) Enzyme-labeled streptoavidin solution

Mix 60 μL of the enzyme-labeled streptoavidin stock solution with 12 mL of the enzyme-labeled streptoavidin

diluent. Prepare the volume required just before the third reaction and use promptly.

(5) Substrate solution

Mix 6 mL of substrate solution A and 6 mL of substrate solution B. Prepare the volume required just before the coloring reaction and use promptly.

3. Operating procedure

The operating procedure is outlined in Fig. 2.

(1) Specimen dilution

Mouse serum

1) Let the temperature of the necessary constituent reagents return to 22 – 28 ºC.
2) Mix 10 µL of serum with 1mL of the specimen diluent solution (preparation of 101-fold-diluted specimen).
3) Mix 10µL of 101-fold-diluted specimen prepared in above 2) with 1.0 mL of the specimen diluent solution (preparation of 10,201-fold-diluted specimen).

Rat serum

1) Let the temperature of the necessary constituent reagents return to 22 – 28 ºC.
2) Mix 10 µL of serum with 1.0 mL of the specimen diluent solution (preparation of 101-fold-diluted specimen).
3) Mix 100µL of 101-fold-diluted specimen prepared in above 2) with 1.0 mL of the specimen diluent solution (preparation of 1,111-fold-diluted specimen).

Adipocyte extract or culture supernatant

1) Let the temperature of the necessary constituent reagents return to 22 – 28 ºC.
2) Mix 10 µL of adipocyte extract or culture supernatant with 1mL of the specimen diluent solution (preparation of 101-fold-diluted specimen).
3) Mix 50µL of 101-fold-diluted specimen prepared in above 2) with 1mL of the specimen diluent solution (preparation of 2,121-fold-diluted specimen).

(2) Measuring procedure

1) Let the temperature of the respective constituent reagents return to 22 – 28 ºC.
2) Prepare the washing solution, specimen diluent, and the working standard solutions of the respective concentrations.
3) Open the aluminum-laminated bag of the antibody plate and take out the number of strips required for the test. Check the positions of the standard solutions and the specimen with the data sheet, plate map, etc.
4) Add approximately350 µL of the washing solution to each well of the antibody plate and remove the solution in the wells completely by aspiration with the plate washer. Then, turn the antibody plate upside down, and pat the plate lightly on a paper towel, etc. to remove the residual washing solution in the wells.
5) Add 100 µL each of the standard solutions of the respective concentrations and the diluted specimen to the wells. Measure the standard solutions at each measurement and for each antibody plate.
6) Cover the antibody plate with a plate seal and allow it to stand at 22 – 28 ºC for 60 minutes to react.
7) Remove the plate seal from the antibody plate, taking care not to spill the solutions, and remove the solutions in the wells completely by aspiration with the plate washer. Then, add approximately350 µL of the washing solution to each well and remove again promptly by aspiration. Take care not to induce overflowing of the washing solution at this time. Repeat the washing and aspiration procedures two more times and remove the washing solution remaining in the wells as directed in 4).
8) Add 100 µL of the Biotin-labeled antibody solution to each well of the antibody plate.
9) Cover the antibody plate with the plate seal and allow it to react at 22 – 28 ºC for 60 minutes.
10) Wash the wells as directed in 7).
11) Add 100 µL of the enzyme-labeled streptoavidin solution to each well of the antibody plate.
12) Cover the antibody plate with the plate seal and allow it to react at 22 – 28 ºC for 60 minutes.
13) Wash the wells as directed in 7).
14) Add 100 µL of the substrate solution to each well of the antibody plate.
15) Allow reaction at 22 – 28 ºC for 15 minutes, and add 100 µL of the reaction stopper solution to each well of the antibody plate.

16) Measure the absorbance of each well at 450 nm (reference wavelength: 650 nm for measurement at 2 wavelengths) using a plate reader.

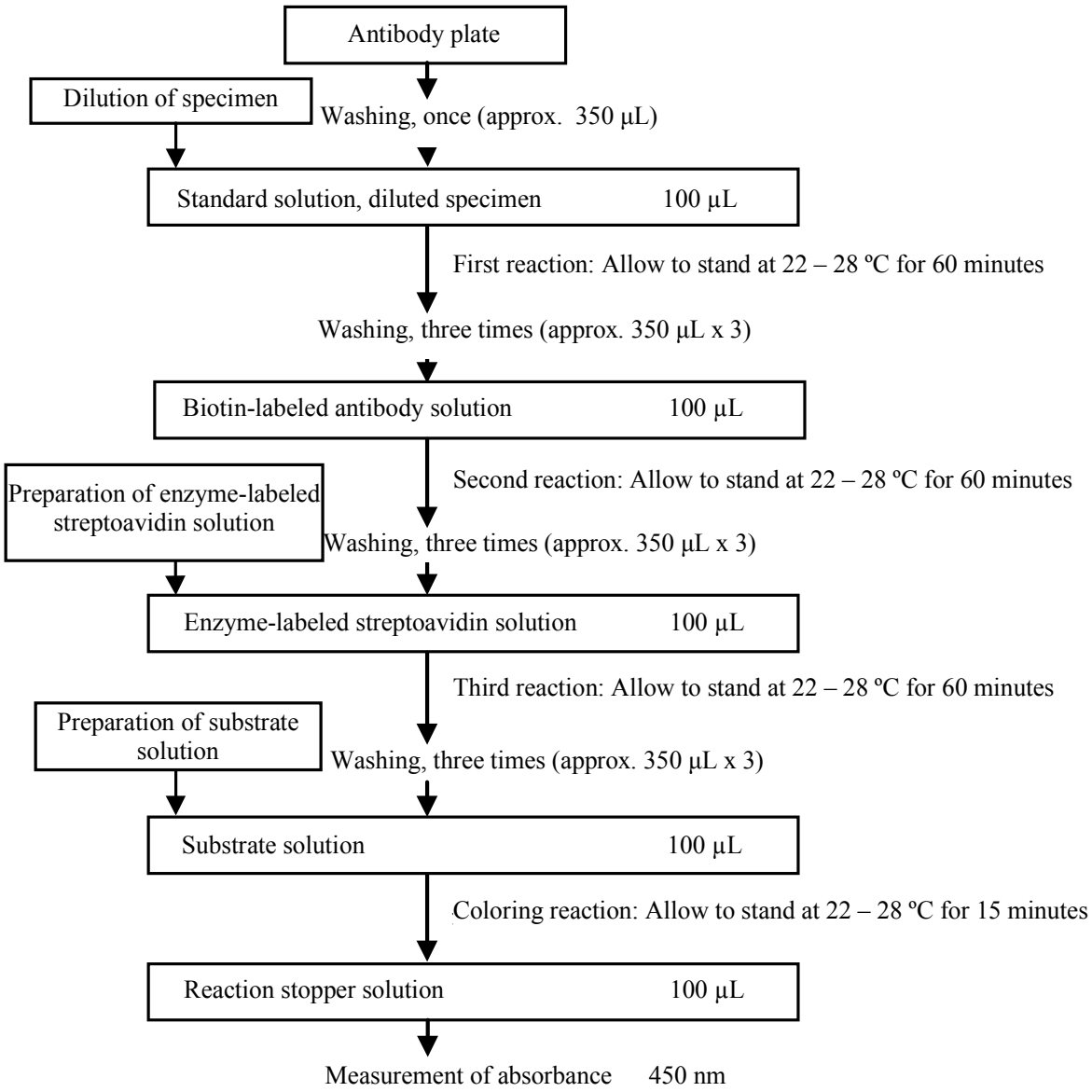

Fig. 2 Outline of the operating procedure

4. Calculation of adiponectin concentration

(1) Calculate the actual absorbances by subtracting the mean absorbance of 0 ng/mL standard solution (blank value) from the absorbances of the respective working standard solutions and the specimen.

(2) Plot the actual absorbances along the Y axis and the concentrations of the working standard solutions along the X axis. Apply an appropriate regression curve to each plot (e.g.: double-logarithmic quadratic regression, etc.; see Fig. 3) and prepare a calibration curve.

(3) Determine the adiponectin concentration of the specimen solution from the calibration curve based on the actual absorbance.

(4) Calculate the adiponectin concentration in the specimen by multiplying the value by the dilution factor (10,201 times for mouse serum, 1,111 times for rat serum, 2,121 times for cell extract or culture supernatant). Rat adiponectin concentration is calculated as the equivalent of mouse adiponectin concentration.

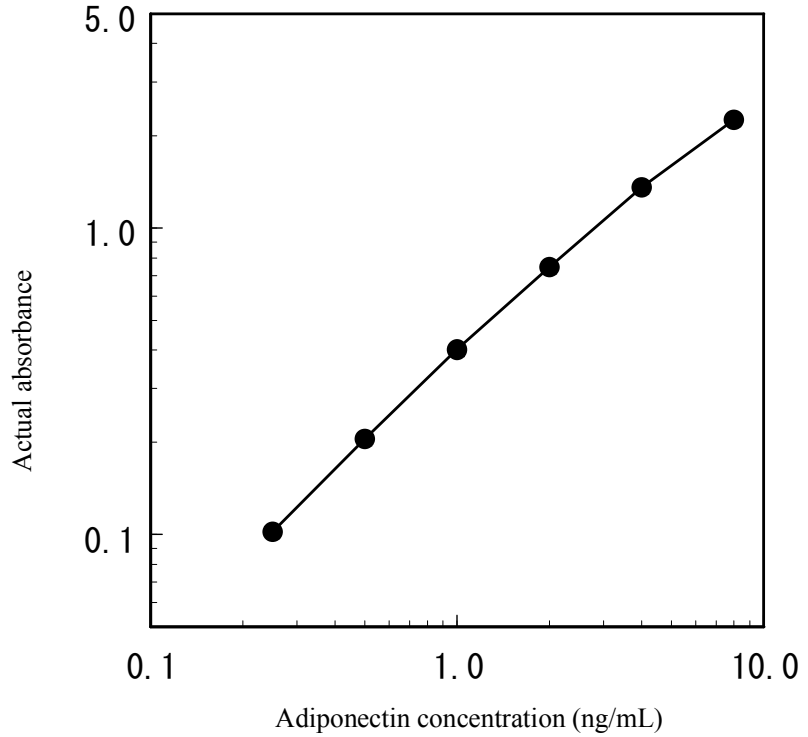

Fig. 3 Example of preparation of a calibration curve by double-logarithmic quadratic regression

[Precautions in operation]

1. Be sure to store the specimen in a freezer (preferably -70 ºC or lower) until measurement.
2. Be sure to store diluted specimen in a freezer (preferably -70 ºC or lower).
3. The values obtained on measurement of various diluted sera (1,111 times) of sheep, pig, cow, and cow fetus using the kit were beneath the lower limit (0.25 ng/mL) of the measurement range.
4. Use the respective constituent reagents after returning them to 22 – 28 ºC and mixing well. If crystals have precipitated in the stock washing solution or specimen pretreatment solution, warm to dissolve them before use.
5. Prepare the reagents at the appropriate times as directed previously. Particular attention is needed in preparation of the substrate solutions, to prevent increase in the blank value and formation of suspended solids (crystals) over time after preparation.
6. The kit may be used in 4 tests, provided that the residual constituent reagents are stored in airtight vessels at 2 – 8 ºC. For reuse, return the reagents to 22 – 28 ºC and mix well. Return unused antibody plates to the aluminum-laminated bag together with a desiccant and close tightly, then store at 2 – 8 ºC.
7. Prepare a calibration curve for each antibody plate even if 2 or more kits (antibody plates) of the same manufacturing number are used simultaneously.
8. Do not use kits of different manufacturing numbers in combination.
9. Perform measurement in duplicate, both for the standard solutions and the specimen.
10. Dilute a specimen with the specimen diluent if it contains adiponectin at a high concentration beyond the range of the calibration curve.
11. Take care not to damage the wells during washing and not to dry the wells after washing.
12. Care is needed in distributing the specimen and the reagents to prevent contamination of the specimen or between reagents.
13. Do not use the kit after the expiration date.

[Performance (sensitivity and reproducibility)]

1. Sensitivity test

The absorbance of 8.0 ng/mL standard solution was not less than 1.0.

2. Reproducibility test

The coefficient of variation was less than 10% when both mouse and rat sera with two different concentrations were measured 4 times simultaneously at the manufacturer's laboratory.
The coefficient of variation was less than 15% when both mouse and rat sera with two different concentrations were measured 6 times repeatedly at the manufacturer's laboratory.

3. Measurement range

The kit allows measurement of mouse adiponectin in the range between 0.25 and 8.0 ng/mL. The lower limit of detection was 15.6 pg/mL at the manufacturer's laboratory.

[Precautions for use or handling]

1. Do not use the constituent reagents of the kit for purposes other than measurement of adiponectin.
2. Do not orally aspirate the pipettes used for sampling.
3. Handle the standard solutions and specimens carefully, since they are always associated with a risk of infection. Handle apparatus such as tips coming into contact with a specimen or residual specimen solutions and their vessels similarly.
4. Handle the reaction stopper solution carefully in order to prevent contact with the skin, etc. since this solution contains sulfuric acid.
5. Take emergency action such as thorough washing with water if the reagents come in contact with the eyes or mouth or skin, and seek the assistance of a physician, if necessary.
6. Incinerate the vessels and pipettes used or dispose of them by separating medical waste and industrial waste in accordance with regulations concerning waste.
7. Dispose of the kit carefully with large volumes of water, since it includes constituent reagents containing sodium azide (0.1 w/v% or less)(specimen stock diluent, 8.0 ng/mL reference standard, and biotin-labeled antibody solution).
8. Sterilize apparatus such as tips coming into contact with the standard solutions, specimens, or residual solutions, as well as their containers by autoclaving (121 ºC, 20 minutes) or by immersion in sodium hypochlorite solution (effective chlorine concentration: 1000 ppm or higher) for more than 1 hour, since they are associated with a risk of infection.
9. This kit is intended for research purposes use only, not for diagnosis or its aid.

**[Storage condition and expiration date]

2 – 8 ºC, for 18 months from the date of manufacturing (expiration date is indicated on the package)

[Packaging unit]

For 96 tests

[References]

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