

Reagent for research purposes only

\*\* Revised: April, 2006  
\* Revised: December, 2005

## Human 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]

Adiponectin is a cytokine (adipocytokine) identified by the group of Matsuzawa et al., Department of Internal Medicine and Molecular Science, Osaka University, in 1996 as a genetic apM1 (adipose most abundant gene transcript) expressed specifically in adipose tissues, and is a secretory protein consisting of 244 amino acids. It has been reported that it forms multimers in blood and has a structure homologous to type VIII and X collagens and also has a spherical structure homologous to complement component C1q.<sup>1,2)</sup> Adiponectin has recently been reported to exhibit various physiological activities; in particular, its inhibition of smooth muscle cell proliferation and of adhesion of monocyte to endothelial cells is expected to inhibit arteriosclerosis.<sup>3,4,5)</sup> Moreover, it was found in a study in mice that administration of adiponectin improved diabetes mellitus.<sup>6)</sup> It is known that adiponectin are present in great abundance in human blood ( $\mu\text{g/mL}$ ), and relationships between decrease in serum level of it and obesity<sup>7)</sup>, diabetes mellitus<sup>8,9)</sup>, and arteriosclerosis<sup>4,8)</sup> have been reported.

This kit is based on the enzyme-linked immunosorbent assay (ELISA) specific to human adiponectin developed in a cooperative study with Matsuzawa, et al., and a recombinant human adiponectin is used as the reference standard. This kit enables measurement of adiponectin in human serum or plasma 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	Specimen pretreatment solution	10 mL/bottle	Buffer, etc.
3	Stock specimen diluent	50 mL/bottle	Buffer, etc.
4	Antibody plate	96 wells/plate	Anti-human adiponectin monoclonal antibody, etc.
5	12 ng/mL reference standard	2 mL/tube	Recombinant human adiponectin, etc.
6	Primary antibody solution	12 mL/bottle	Anti-human adiponectin antiserum (rabbit), etc.
7	Enzyme-labeled antibody stock solution	0.1 mL/tube	Horseradish peroxidase-labeled anti-rabbit IgG polyclonal antibody (goat), etc.
8	Enzyme-labeled antibody diluent	15 mL/bottle	Buffer, etc.
9	Substrate solution A	7.5 mL/bottle	3,3',5,5'-tetramethylbenzidine, etc.
10	Substrate solution B	7.5 mL/bottle	Hydrogen peroxide, etc.
11	Reaction stopper solution	15 mL/bottle	Sulfuric acid, etc.

#### \*\* [Packaging]

Packaging unit	Quantity	Accessory
For 96 tests	1 set of constituting reagents (1 to 11)	6 plate seals
For 480 tests	5 sets of constituent reagent (1 to 11)	24 plate seals

#### [Purpose of use]

Measurement of adiponectin in human serum or plasma as well as in adipocyte extract or culture supernatant

### [Principle of measurement]

This product is a kit for measurement of human adiponectin using ELISA.

The principle of measurement is shown in Fig. 1. When a specimen or the standard solution pretreated in advance is added to a plate immobilized with anti-human adiponectin monoclonal antibody to allow reaction, adiponectin binds to the antibody plate (first reaction). The anti-human adiponectin antiserum (rabbit) (primary antibody solution) is allowed to react (second reaction), followed by reaction with the horseradish peroxidase-labeled anti-rabbit IgG polyclonal antibody (goat) (enzyme-labeled antibody 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.

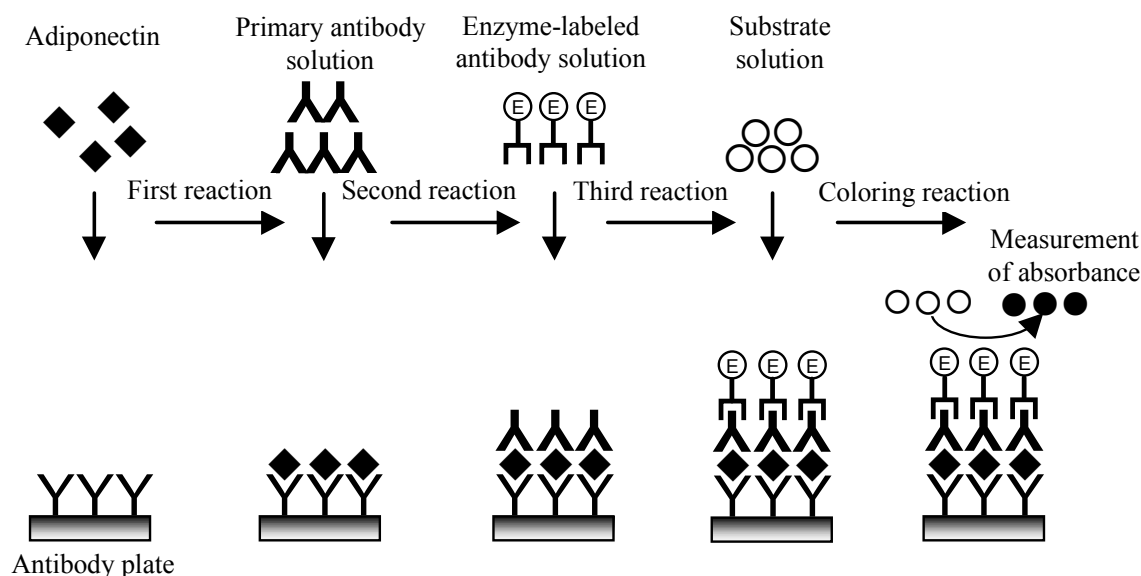


Fig. 1. Principle of Measurement

### [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) Heat block, etc.
- (8) 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 °C 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 °C after preparation.

##### (3) Standard solution

Dilute the 12 ng/mL reference standard stepwise with the specimen diluent to obtain working standard solutions of 6.0, 3.0, 1.5, 0.75, and 0.38 ng/mL. Use the 12 ng/mL reference standard as the standard solution of 12 ng/mL and

the specimen diluent as the standard solution of 0 ng/mL.

**(4) Enzyme-labeled antibody solution**

Mix 60  $\mu$ L of the enzyme-labeled antibody stock solution with 12 mL of the enzyme-labeled antibody diluent. Prepare the volume required just before the second 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. After obtaining the required amount, cap the vial containing substrate solution A immediately and store at 2 – 8 °C.

**\*(6) Specimen pretreatment solution**

If crystals have precipitated in the specimen pretreatment solution, warm the solution to dissolve them, taking care not to induce frothing.

**3. Operating procedure**

The operating procedure is outlined in Fig. 2.

**(1) Specimen pretreatment**

**Pretreatment of serum or plasma (anticoagulant: EDTA, heparin)**

- 1) Let the temperature of the necessary constituent reagents return to 20 – 30 °C.
- 2) Mix 10  $\mu$ L of a serum or plasma (anticoagulant: EDTA, heparin) with 90  $\mu$ L of the specimen pretreatment solution and heat at 100 °C for 5 minutes using a heating block, etc. Use a capped microtube or similar container of 1.5 mL or more in volume for heating (preparation of 10-fold-diluted specimen).
- 3) Add 900  $\mu$ L of the specimen diluent and mix (preparation of 100-fold-diluted specimen).
- 4) Transfer 20  $\mu$ L of the 100-fold-diluted serum in 3) to a clean container and mix with 1.0 mL of the specimen diluent (5100-fold-diluted specimen).

**Pretreatment of adipocyte extract or culture supernatant**

- 1) Let the temperature of the necessary constituent reagents return to 20 – 30 °C.
- 2) Dilute the specimen pretreatment solution 5-fold with purified water (5-fold-diluted specimen pretreatment solution).
- 3) Mix 10  $\mu$ L of the adipocyte extract or culture supernatant with 90  $\mu$ L of the 5-fold-diluted specimen pretreatment solution prepared in 2) and heat at 100 °C for 5 minutes using a heating block, etc. Use a capped microtube or similar container of 1.5 mL or more in volume for heating (preparation of 10-fold-diluted specimen).
- 4) Add 900  $\mu$ L of the specimen diluent and mix (preparation of 100-fold-diluted specimen).

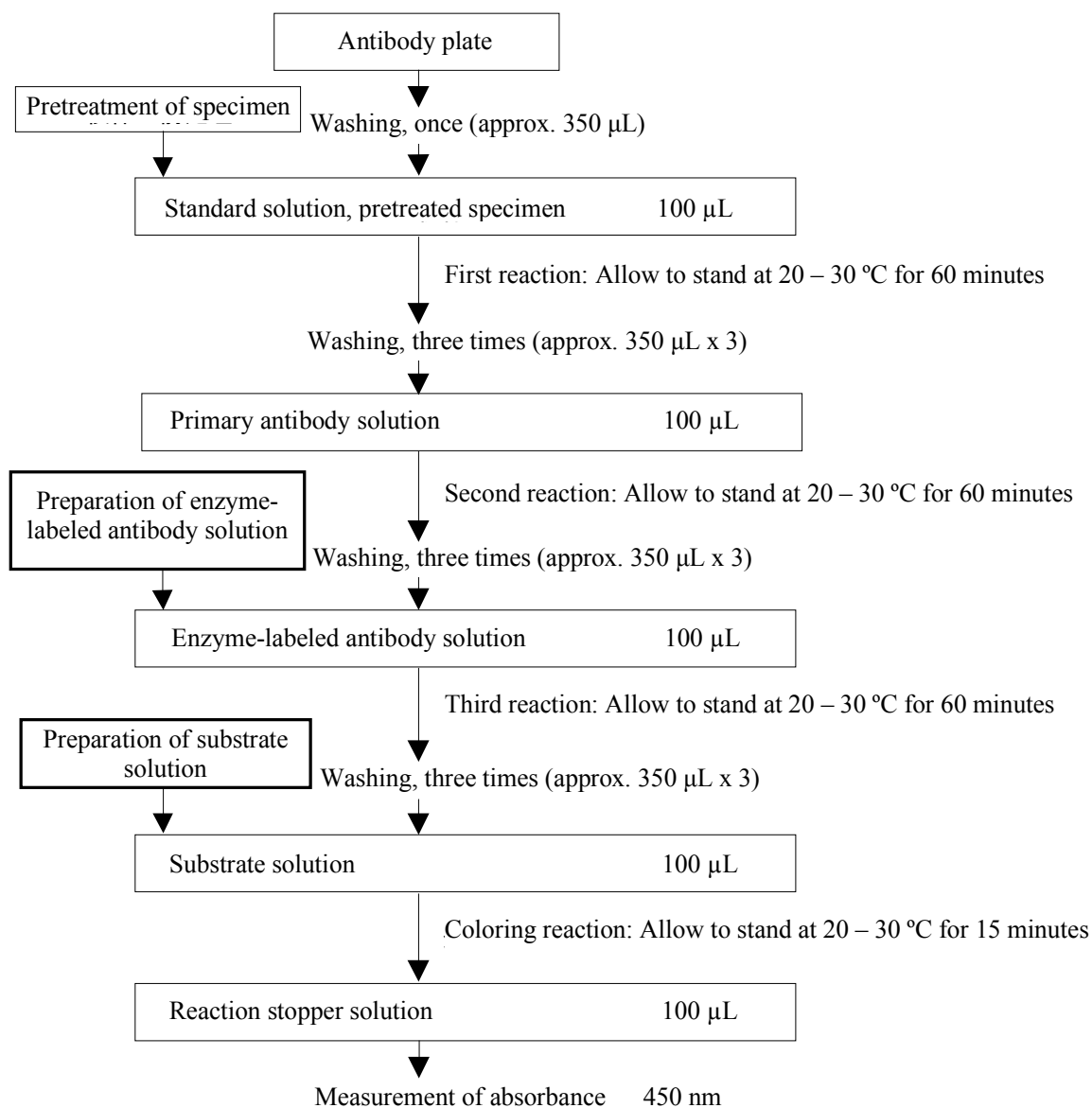


Fig. 2 Outline of the operating procedure

## (2) Measuring procedure

- 1) Let the temperature of the respective constituent reagents return to 20 – 30 °C.
- 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 approximately 350 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 pretreated 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 20 – 30 °C 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 approximately 350  $\mu\text{L}$  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  $\mu\text{L}$  of the primary antibody solution to each well of the antibody plate.
- 9) Cover the antibody plate with the plate seal and allow it to react at 20 – 30  $^{\circ}\text{C}$  for 60 minutes.
- 10) Wash the wells as directed in 7).
- 11) Add 100  $\mu\text{L}$  of the enzyme-labeled antibody solution to each well of the antibody plate.
- 12) Cover the antibody plate with the plate seal and allow it to react at 20 – 30  $^{\circ}\text{C}$  for 60 minutes.
- 13) Wash the wells as directed in 7).
- 14) Add 100  $\mu\text{L}$  of the substrate solution to each well of the antibody plate.
- 15) Allow reaction at 20 – 30  $^{\circ}\text{C}$  for 15 minutes, and add 100  $\mu\text{L}$  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.

#### **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 (5100 times for serum or plasma, 100 times for cell extract or culture supernatant).

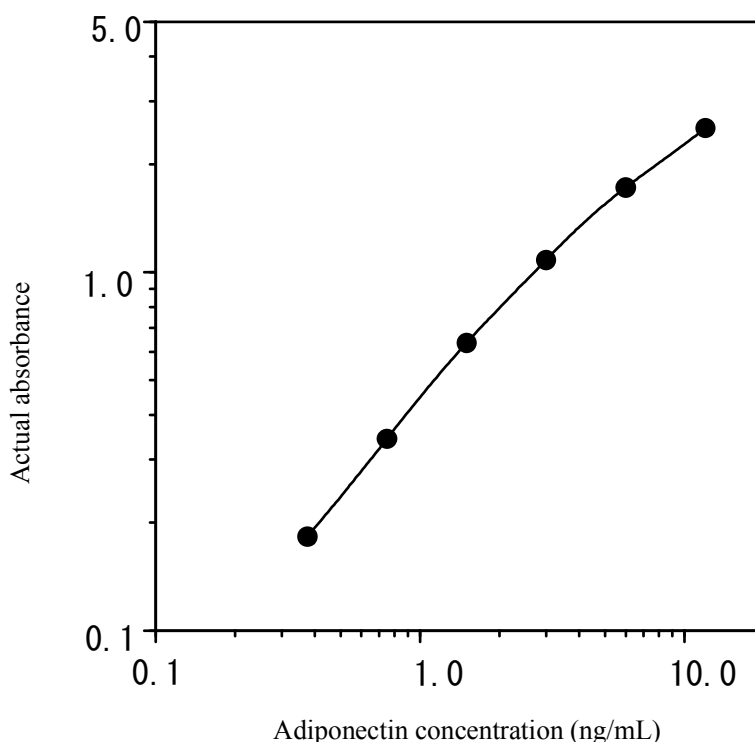


Fig. 3 Example of preparation of a calibration curve by double-logarithmic quadratic regression

#### **[Precautions in operation]**

1. Be sure to store the specimen in a freezer (preferably -70 °C or lower) until measurement.
2. Use plasma treated with EDTA or heparin as specimens. Do not use citrate-treated plasma, which tend to exhibit incorrectly lower values on measurement.
3. Be sure to store pretreated specimen in a freezer (preferably -70 °C or lower).
4. Cross-reaction with a recombinant mouse adiponectin was absent even at 320 ng/mL. The values obtained on measurement of various sera of mouse, rat, goat, sheep, pig, cow, and cow fetus using the kit were beneath the lower limit (0.38 ng/mL) of the measurement range.
5. Use the respective constituent reagents after returning them to 20 – 30 °C and mixing well. If crystals have precipitated in the stock washing solution or specimen pretreatment solution, warm to dissolve them before use.
6. 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.
7. The kit may be used in 4 tests, provided that the residual constituent reagents are stored in airtight vessels at 2 – 8 °C. For reuse, return the reagents to 20 – 30 °C and mix well. Return unused antibody plates to the aluminum-laminated bag together with a desiccant and close tightly, then store at 2 – 8 °C.
8. Prepare a calibration curve for each antibody plate even if 2 or more kits (antibody plates) of the same manufacturing number are used simultaneously.
9. Do not use kits of different manufacturing numbers in combination.
10. Perform measurement in duplicate, both for the standard solutions and the specimen.
11. Dilute a specimen with the specimen diluent if it contains adiponectin at a high concentration beyond the range of the calibration curve.
12. Take care not to damage the wells during washing and not to dry the wells after washing.
13. Care is needed in distributing the specimen and the reagents to prevent contamination of the specimen or between reagents.
14. Do not use the kit after the expiration date.

#### **[Performance (sensitivity and reproducibility)]**

##### **1. Sensitivity test**

The absorbance of 12 ng/mL standard solution was not less than 1.0.

## 2. Reproducibility test

The coefficient of variation was less than 10% when specimens with two different concentrations were measured 4 times simultaneously at the manufacturer's laboratory.

The coefficient of variation was less than 10% when specimens with two different concentrations were measured 6 times repeatedly at the manufacturer's laboratory.

## 3. Measurement range

The kit allows measurement of adiponectin in the range between 0.38 and 12 ng/mL. The lower limit of detection was 23.4 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 human 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, 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, 12 ng/mL reference standard, and primary 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 °C, 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 °C, for 12 months from the date of manufacturing (the expiration date is indicated on the package)

## \*\*[Packaging unit]

For 96 tests and 480 tests

## [References]

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- 2) Maeda K, et al.: Gene, 190, 227-235, 1997
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- 9) Hotta K, et al.: Diabetes, 50, 1126-1133, 2001

## [Manufacturer]

Otsuka Pharmaceutical Co., Ltd.

## [Distributor]

**SCETI** SCETI K.K.  
3-6-7, Kasumigaseki, Chiyoda-ku  
Tokyo 100-0013 Japan  
Tel: +81-3-5510-2347 Fax: +81-3-5510-0133  
URL: <http://www.sceti.co.jp/export/>  
Email: [exp-pet@sceti.co.jp](mailto:exp-pet@sceti.co.jp)