Reagent for research purposes only

*Revised: September 2009 (Third Edition)
Revised: June 2008 (Second Edition)

Human High-Molecular-Weight (HMW) Adiponectin ELISA Kit
Instructions for use

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

[Background of development and characteristics]

Adiponectin is an adipocytokine identified by the group of Matsuzawa et al., Department of Internal Medicine and Molecular Science, Osaka University, in 1996, which is a secretory protein consisting of 244 amino acids. It has been reported that it forms multimers[1] in blood and has a structure homologous to type VIII and X collagens and a spherical structure homologous to complement component C1q.[2, 3] Studies have reported that adiponectin inhibits smooth-muscle cell proliferation and adhesion of monocytes to endothelial cells and thereby inhibits arteriosclerosis,[4] that it is also a regulator of insulin sensitivity,[5] and that it is a molecule playing a key role in the pathogenesis of metabolic syndrome.[6] Adiponectin in the blood consists of low-molecular-weight, middle-molecular-weight, and high-molecular-weight fractions.[7] Since the change in blood concentration of adiponectin is reported to be largely due to the change in the concentration of high-molecular-weight fractions,[8] the significance of the measurement thereof has received attention.

This kit has been developed for use in enzyme-linked immunosorbent assays (ELISA) for the specific quantitative determination of high-molecular-weight adiponectin in human blood.

[Composition of the kit]

	Constituent reagent	Volume/Quantity etc.	Ingredient, etc.
1	Stock washing solution	40 mL x 1 bottle	Buffer, etc.
2	Stock specimen diluent	50 mL x 1 bottle	Buffer, etc.
3	Antibody plate	96 wells x 1 plate	Anti-human adiponectin monoclonal antibody, etc.
5	Biotin-labeled antibody stock solution	0.1 mL x 1 tube	Biotin-labeled anti-human adiponectin antibody, etc.
6	Biotin-labeled antibody diluent	15 mL x 1 bottle	Buffer, etc.
7	Enzyme-labeled streptavidin stock solution	0.1 mL x 1 tube	Horseradish peroxidase-labeled streptavidin, etc.
8	Enzyme-labeled streptavidin diluent	15 mL x 1 bottle	Buffer, etc.
9	Substrate solution A	7.5 mL x 1 bottle	3,3',5,5'-tetramethylbendizine, etc.
10	Substrate solution B	7.5 mL x 1 bottle	Hydrogen peroxide, etc.
11	Reaction stopper solution	15 mL x 1 bottle	Sulfuric acid, etc.

Separate package (Keep frozen: store at -20ºC or lower)

4	200 ng/mL reference standard	0.5 mL x 4 tubes	Pooled serum etc.

Accessory: Plate seal x 6 pcs

[Purpose of use]

Measurement of high-molecular-weight adiponectin in human serum or heparin sodium plasma

[Principle of measurement]

This product is a kit for the measurement of human high-molecular-weight adiponectin using ELISA.

The principle of measurement is shown in Figure 1. When a specimen or the standard solution diluted in advance is added to a plate immobilized with anti-human adiponectin monoclonal antibody to allow reaction, high-molecular-weight adiponectin binds to the Antibody plate (first reaction). The plate is reacted with biotin-labeled antibody (second reaction) and further with enzyme-labeled streptavidin (third reaction). Then, a substrate solution is added to develop color (coloring reaction), and the concentration of high-molecular-weight adiponectin in the specimen is calculated from the absorbance measured at 450 nm and the absorbance of the standard solution measured simultaneously.

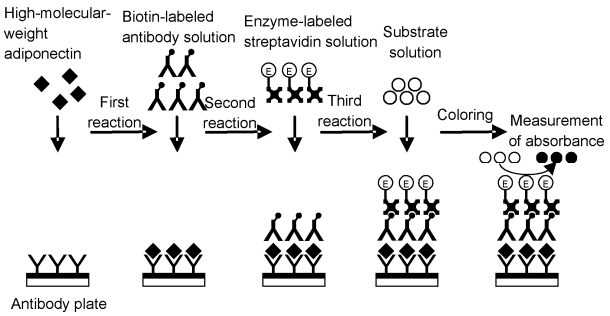

Figure 1 Principle of measurement

[Method of Measurement]

1. Apparatus, instruments, etc.

(1) Measuring cylinder
(2) Measuring pipette
(3) Micropipette and tip
(4) Plate washer
(5) Paper towel
(6) Plate reader (measuring wavelength: 450 nm)

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 – 8ºC after preparation.

(2) Specimen diluent
Mix the whole volume (50 mL) of the Stock specimen diluent with 200 mL of purified water. If crystals have precipitated in the stock specimen diluent, dissolve them by warming before preparation. Store the solution at 2 – 8 ºC after preparation.

(3) Standard solutions
Melt 200 ng/mL of reference standard at room temperature, stir on a vortex mixer, and serially dilute in two-fold steps with the specimen diluent to prepare

working standard solutions of 100, 50.0, 25.0, 12.5, 6.25, and 3.13 ng/mL.
Use the 200 ng/mL reference standard as the standard solution of 200 ng/mL and the specimen diluent as the standard solution of 0 ng/mL.
Store the residue of the 200 ng/mL reference standard at 2 – 8ºC and use within one week. The residue may be used later than one week after the melting if it is refrozen; freezing and melting may not be repeated more than three times.

(4) Biotin-labeled antibody solution
Mix 60 µL of the Biotin-labeled antibody stock solution with 12 mL of the Biotin-labeled antibody diluent. Prepare the volume required just before the second reaction and use promptly.

(5) Enzyme-labeled streptavidin solution
Mix 60 µL of the Enzyme-labeled streptavidin stock solution with 12 mL of the Enzyme-labeled streptavidin diluent. Prepare the volume required just before the third reaction and use promptly.

(6) 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 – 8ºC.

3. Operating procedure

The operating procedure is outlined in Figure 2.

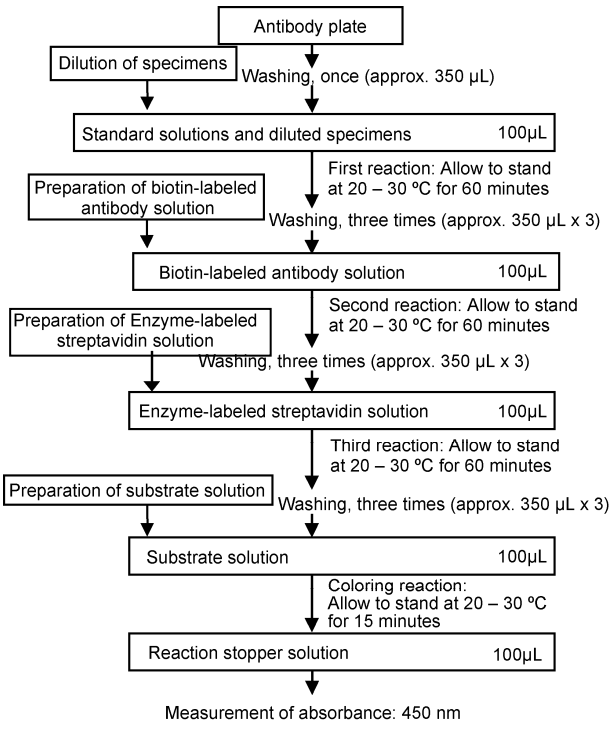

Figure 2 Outline of the operating procedure

(1) Dilution of specimens

Dilute serum or plasma obtained with the heparin sodium blood collection tube

1) Let the temperature of the necessary constituent reagents return to 20 – 30ºC.
2) Mix 10 µL of a serum or plasma (heparin) with 1000 µL of the specimen diluent.

(2) Measuring procedure

1) Let the temperature of the respective constituent reagents return to 20 – 30ºC.
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 each well 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 or the diluted specimen to each well. 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 – 30ºC 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 each well completely by aspiration with the plate washer. Then, add approximately 350 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 each well 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 a plate seal and allow it to stand at 20 – 30ºC for 60 minutes to react.
10) Wash the wells as directed in (7)).
11) Add 100 µL of the enzyme-labeled streptavidin solution to each well of the Antibody plate.
12) Cover the Antibody plate with a plate seal and allow it to stand at 20 – 30ºC for 60 minutes to react.
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 20 – 30ºC 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.

4. Calculation of high-molecular-weight 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 Figure 3) and prepare a calibration curve.

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

(4) Calculate the high-molecular-weight adiponectin concentration in the specimen by multiplying the value by the dilution factor (101 times for serum or plasma).

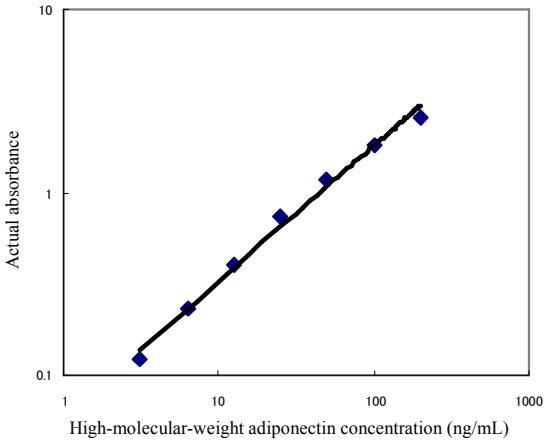

Figure 3 Example of the calibration curve prepared with double-logarithmic quadratic regression

[Precautions in operation]

1. Be sure to store the specimen in a freezer (preferably -70ºC or lower) until measurement.
2. The only human biological components that may be used as specimens are serum and plasma obtained with heparin sodium blood collection tubes. Plasma obtained with other blood collection tubes may not be used because the values measured for these plasmas tend to be low.
3. Be sure to store diluted specimen in a freezer (preferably -70ºC or lower).
4. The values obtained on measurement of various sera of rabbit, cow, sheep, goat, chicken, cat, hamster, horse, and rat using the kit were below the lower limit (3.13 ng/mL) of the measurement range.
5. Use the respective constituent reagents after returning them to 20 – 30ºC and mixing well. If crystals have precipitated in the Stock washing solution or Stock specimen diluent, 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. Store the 200 ng/mL reference standard in a freezer (at –20ºC or lower) until needed and melt at room temperature before using for preparing standard solutions. Store the residue in opened packages at 2 – 8ºC and use within one week. The residue may be stored and used later than one week after melting if it is refrozen; freezing and melting may not be repeated more than three times. On the other hand, in the case of standard solutions obtained by stepwise dilution, do not use stored solutions, but prepare the required volumes every time before use.
8. The kit may be used separately for up to four tests. After separated use, the residual constituent reagents should be stored in airtight vessels at 2 – 8ºC. Use the reference standard within one week. For reuse, return the reagents to 20 – 30ºC and mix well. Return unused Antibody plates to the aluminum-laminated bag together with a desiccant and close tightly, then store at 2 – 8ºC.
9. Prepare a calibration curve for each Antibody plate if 2 or more kits (Antibody plates) of the same manufacturing number are used simultaneously.
10. Do not use kits of different manufacturing numbers in combination.
11. Perform measurement in duplicate, both for the standard solutions and the specimen.
12. Dilute a specimen with the diluent if it contains adiponectin at a high concentration beyond the range of the calibration curve.
13. Take care not to damage the wells during washing and not to dry the wells after washing.
14. Care is needed in distributing the specimen and the reagents to prevent contamination of the specimen or between reagents.
15. Do not use the kit after the expiration date.

[Performance (sensitivity and reproducibility)]

1. Sensitivity test
   The absorbance of 200 ng/mL standard solution was not less than 1.0.
2. Reproducibility test
   The coefficient of variation was less than 10% when specimens with three different concentrations were measured 4 times simultaneously at the manufacturer's laboratory.
   The coefficient of variation was less than 10% when specimens with three different concentrations were measured 5 times repeatedly at the manufacturer's laboratory.
3. Measurement range
   High-molecular-weight adiponectin concentrations in the range of 3.13 – 200 ng/mL can be measured.

[Precautions for use or handling]

1. Reagents constituting this kit may not be used for purposes other than the measurement of human high-molecular-weight 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. Sterilize apparatus such as tips coming into contact with the standard solutions, specimens, or residual solutions, as well as their containers by autoclaving (121ºC, 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.
8. Dispose of the kit carefully with large volumes of water, since it includes constituent reagents containing sodium azide (0.1 w/v% or less) (Stock specimen diluent and 200 ng/mL reference standard).
9. This kit is intended for research purpose use only, not for diagnosis or its aid.

***[Storage condition and expiration date]**

Kit: at 2 – 8ºC, Reference standard (separately packaged): at -20ºC or lower

Expiration date: 12 months from the date of manufacturing (the expiration date is indicated on the package)

[Packaging unit]

For 96 tests

[References]

1) Waki H, et al. : J Biol Chem, 278, 40352-40363, 2003
2) Maeda K, et al. : Biochem Biophys Res Commun, 221, 286-289, 1996
3) Maeda K, et al. : Gene, 190, 227-235, 1997
4) Ouchi N, et al. : Circulation, 102, 1296-1301, 2000
5) Tonelli J, et al. : Diabetes, 53, 1621-1629, 2004
6) Hara K, et al. : Diabetes Care, 29 1357-1362, 2006
7) Nakano Y, et al. : J Lipid Res, 47 1572-1582, 2006
8) Kobayashi H, et al. : Circ Res, 94 e27-31, 2004

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, BELGIUM
Tel 0032 16 58 90 45 | Fax 0032 16 50 90 45
www.gentaur-worldwide.com
info@gentaur.com