KAMIYA BIOMEDICAL COMPANY

Human High Molecular Weight Adiponectin (HMWA) ELISA Kit

For the quantitative determination of HMWA in human serum or heparin sodium plasma.

Cat. No. KT-601

For Research Use Only.

PRODUCT INFORMATION

Human High Molecular Weight Adiponectin (HMWA) ELISA Kit

Cat. No. KT-601

INTENDED USE

The Human HMWA EIA is for the quantitative determination of HMWA in serum or heparin sodium plasma. For research use only.

INTRODUCTION

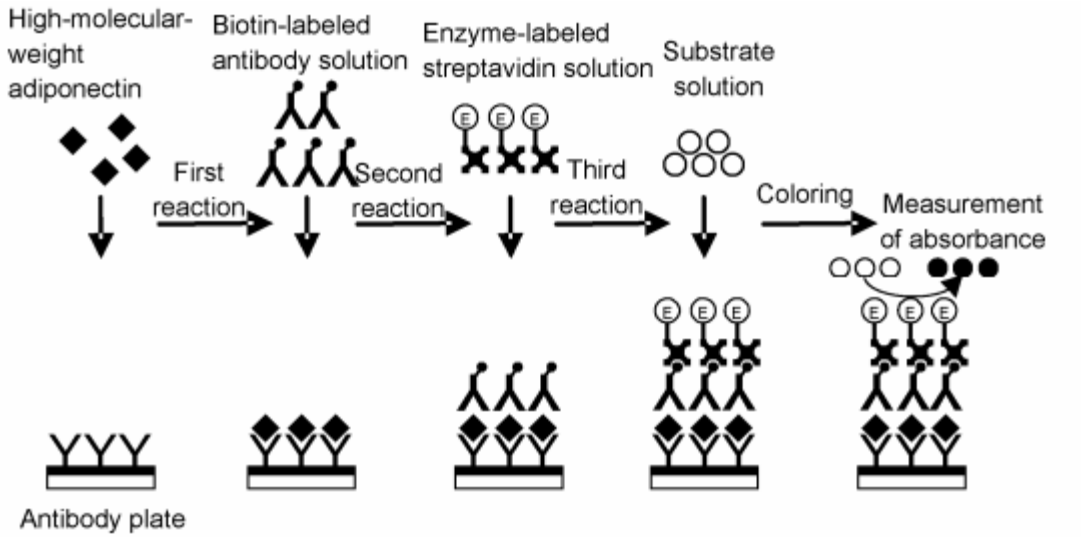
Adiponectin is an adipocytokine which is a secretory protein consisting of 224 amino acids. It has been reported that it forms multimers in blood and has a structure homologous to type VIII and X collagens and a spherical structure homologous to complement component C1q. Studies have reported that adiponectin inhibits smooth-muscle cell proliferation and adhesion of monocytes to endothelial cells and thereby inhibits arteriosclerosis, that it is also a key regulator of insulin sensitivity, and that it is a molecule playing a key role in the pathogenesis of metabolic syndrome. Adiponectin in the blood consists of low-molecular-weight, middle-molecular-weight, and high-molecular-weight fractions. 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, the significance of the measurement thereof has received attention.

COMPONENTS

Reagent	Volume	Ingredient, ect.
Wash Solution	40 mL	Buffer
Stock Sample Diluent	50 mL	Buffer
Antibody Plate	96 wells	Anti-human adiponectin antibody
Calibrator 200 ng/mL	0.5 mL x 4	Pooled serum
Biotin Labeled Antibody Solution	0.1 mL	Biotin labeled anti-human adiponectin antibody
Biotin Labeled Antibody Diluent	15 mL	Buffer
Enzyme Labeled Streptoavidin	0.1 mL	Horseradish peroxidase streptoavidin
Enzyme Labeled Streptoavidin Diluent	15 mL	Buffer
Substrate Solution A	7.5 mL	3, 3', 5, 5'- tetramethylbendizine
Substrate Solution B	7.5 mL	Hydrogen peroxide
Stop Solution	15 mL	Sulfuric Acid
Plate Seals	6	

PRINCIPLE

This product is a kit for measurement of human HMWA using ELISA. The principle of measurement is shown below. When a sample or calibrator solution is added to a pre-coated plate with anti-human adiponectin antibody and allowed to react, HMWA binds to the antibody plate. The biotin-labeled antibody is allowed to react, followed by reaction with the HRP-labeled streptoavidin. As substrate solution is added to develop the color and the concentration of HMWA in the sample is calculated from the absorbance measured at 450 nm and the absorbance of the calibrators is measured simultaneously.



MATERIALS REQUIRED BUT NOT PROVIDED

- Graduated cylinder
- Measuring pipette
- Micropipette and tip
- Plate washer
- Paper towels
- Plate reader (450 nm)

PREPARATION

Wash Buffer- Mix whole volume (40 mL) of the stock washing solution with 960 mL of deionized water. If crystals have precipitated in the stock washing solution, dissolve them by warming before preparation. Store the solution at 4℃ after preparation.

Sample Diluent- Mix whole volume (50 mL) of the stock sample diluent with 200 mL of deionized water. Store solution at 4℃ after preparation.

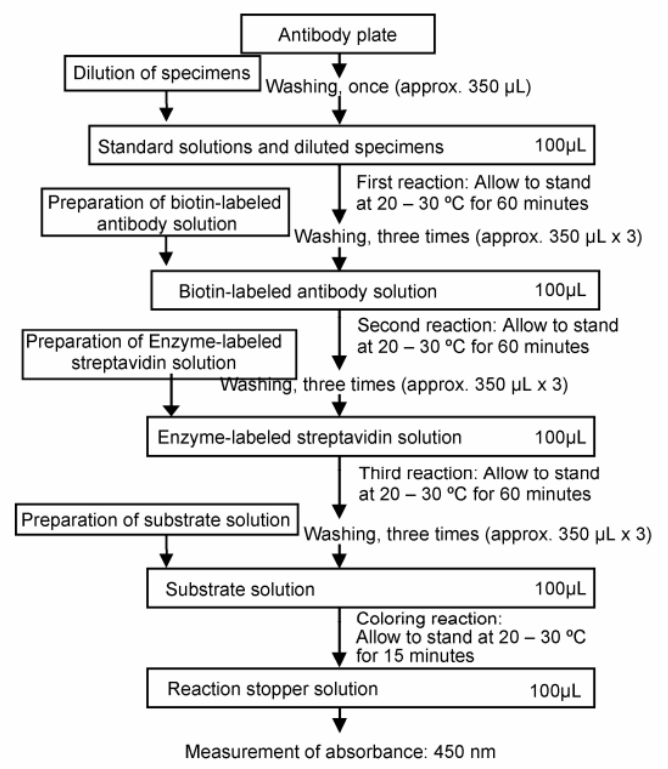
Calibrator- Allow calibrator to reach room temperature, centrifuge before use. Dilute the 200 ng/mL calibrator solution stepwise with the sample diluent to obtain working solutions of 100, 50, 25, 12.5, 6.25 and 3.13 ng/mL. Use the 200 ng/mL calibrator as the 200 ng/mL calibrator and the sample diluent as the 0 ng/mL calibrator.

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.

Enzyme Labeled Streptavidin- mix 60 µL of the enzyme labeled streptavidin solution with 12 mL of the enzyme labeled streptavidin diluent. Prepare the volume required just before the third reaction and use promptly.

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 4℃.

ASSAY PROCEDURE



Sample Dilution

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

1. Let the temperature of the necessary constituent reagents reach room temperature.
2. Mix 10 µL of a serum or heparin sodium plasma sample with 1 mL of sample diluent.

Procedure

1. Let the temperature of the reagents reach room temperature.
2. Prepare the washing solution, sample diluent, and the calibrators of respective concentrations.
3. Open the aluminum laminate bag of the antibody plate and take out the number of strips required for the test.
4. Add approximately 350 µL of the wash solution to each well of the antibody plate and remove the solution in the wells completely by aspiration with plate washer. Then turn the antibody plate upside down and pat the plate lightly on the paper towel to remove the residual wash solution from the wells.
5. Add 100 µL each of the calibrator solutions of respective concentrations and the pretreated samples to the wells. Measure the calibrator solutions at each measurement for the antibody plate.
6. Cover the antibody plate with a plate seal and allow it to incubate at room temperature for 60 minutes.
7. Remove the plate seal and repeat the aspiration/wash process described in step 4 for 3 times.
8. Add 100 µL of the biotin labeled antibody solution to all wells.
9. Cover with a plate seal and incubate for 60 minutes at room temperature.
10. Wash the wells as directed in 7.
11. Add 100 µL of the enzyme labeled streptoavidin to each well of the antibody plate.
12. Cover the antibody plate with a plate seal and allow it to incubate at room temperature for 60 minutes.
13. Wash the wells as directed in 7.

14. Add 100 µL of the substrate solution to all wells.

15. Allow reaction at room temperature for 10 minutes, add 100 µL stop solution to each well of the plate.

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

RESULTS

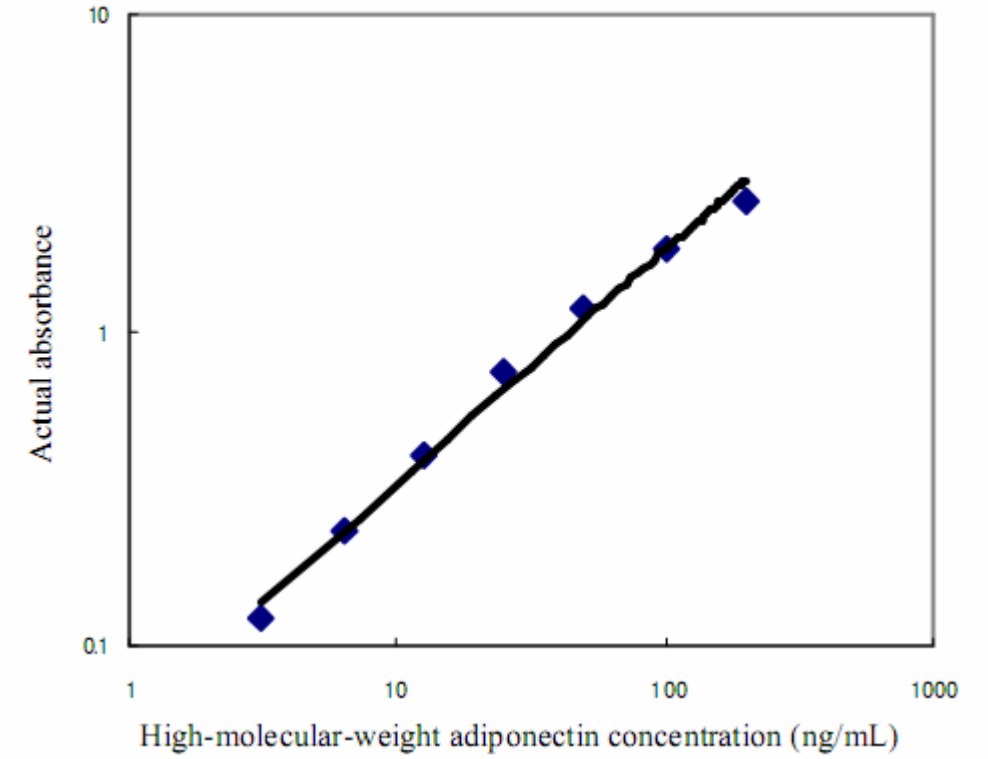
1. Calculate the actual absorbances by subtracting the mean absorbance of the 0 ng/mL calibrator from the absorbances of the samples and calibrators.

2. Plot the actual absorbances along the Y-axis and the concentrations of the working calibrator solutions along the X-axis. Apply an appropriate regression curve to each plot and prepare a calibration curve.

3. Determine the HMWA concentration of the samples from the calibration curve based on the actual absorbance.

4. Calculate the HMWA concentration in the sample by multiplying the value by the dilution factor.

Example of double-logarithmic quadratic regression calibration curve. For example only.



PRECAUTIONS FOR USE

1. Store samples in freezer (preferably -70℃ or lower) until measurement.
2. The values obtained on measurement of various sera of rabbit, sheep, goat, chicken, hamster, horse, cow, and rat using the kit were beneath the lower limit of the measurement range.
3. Use the respective constituent reagents after returning them to room temperature and mixing well. If crystals have precipitated in the stock washing solution or sample pretreatment solution warm to dissolve them before use.
4. Prepare the reagents at the appropriate times as directed previously. Particular attention is needed in the preparation of the substrate solutions, to prevent an increase in the blank value and formation of suspended solids (crystals) over time after preparation.
5. The kit may be used in 4 tests, provided that the residual constituent reagents are stored in airtight vessels at refrigerated temperatures. For reuse, return reagents to room temperature and mix well. Return unused antibody plates to the aluminum laminated bag together with a desiccant and close tightly, then store refrigerated.
6. Do not use kits of different lot numbers in combination.

7. Prepare a calibration curve for each antibody plate even if 2 or more kits (antibody plates) of the same lot number are used simultaneously.
8. Perform measurement in duplicate, both for the calibrator solutions and the samples.
9. Dilute samples with sample diluent if it contains HMWA at a high concentration beyond the range of the calibration curve.
10. Take care not to damage the wells during washing and not to dry the wells after washing.
11. Care is needed in distributing the samples and the reagents to prevent contamination of the samples or between the reagents.
12. Do not use kit after the expiration date.

Performance

Sensitivity test
The absorbance of the 200 ng/mL calibrator was not less than 1.0.

Reproducibility
The coefficient of variation was less than 10% when samples with two different concentrations were measured 4 times simultaneously.

The coefficient of variation was less than 10% when samples with two different concentrations were measured 5 times repeatedly.

Measurement range
The kit allows measurement of HMWA in the range between 3.13 and 200 ng/mL.

PRECAUTIONS FOR HANDLING

1. Do not use the constituent reagents of the kit for purposes other than measurement of human HMWA.
2. Do not orally aspirate the pipettes used for sampling.
3. Handle the calibrator solutions and samples carefully, since they are always associated with a risk of infection. Handle apparatuses such as tips coming into contact with these solutions similarly.
4. Handle the stop 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. 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)(sample stock diluent, 200 ng/mL calibrator, and biotin labeled antibody solution).
8. Sterilize apparatus such as tips coming into contact with the calibrator solutions, samples, or residual solutions as well as their containers by autoclaving (121℃, 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 for research use only. Not for use in diagnostic procedures.

Storage

4℃ until the expiration date listed on the package label. Store calibrator at -20℃.

FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com