KAMIYA BIOMEDICAL COMPANY

Human Obestatin ELISA

For the quantitative determination of obestatin in human serum and plasma

Cat. No. KT-495

For Research Use Only. Not for use in diagnostic procedures.

PRODUCT INFORMATION

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INTENDED USE

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INTRODUCTION

Obestatin is a 23 amino acid residue peptide isolated from rat stomach. The peptide shares the precursor with a food intake stimulating peptide, ghrelin, but possesses reducing effects on food intake, gut motility and body weight. With the use of an antiserum directed against the mouse/rat obestatin, obestatin immunoreactivity (irOBS) was detected in cells of the gastric mucosa and myenteric plexus and in Leydig cells of the testis in Sprague–Dawley rats. Double labeling of myenteric plexus with antisera against obestatin and choline acetyltransferase (ChAT) revealed that nearly all irOBS neurons were ChAT positive and vice versa. Obestatin (100 nM) added to dissociated and cultured rat cerebral cortical neurons elevated cytosolic calcium concentrations [Ca+2]i in a population of cortical neurons. Intracerebroventricular administration of obestatin inhibited water drinking in ad libitum fed and watered rats, and in food and water deprived animals. In addition, obestatin inhibited angiotensin II-induced water drinking in animals provided free access to water and food. Obestatin peptides had no effect on insulin sensitivity as revealed by hypoglycemic response when co-administered with insulin, supporting a role of obestatin in regulating metabolism through changes of appetite, but indicating no direct actions on glucose homeostasis or insulin secretion. It is supposed that in rats the effects of obestatin on food intake may be secondary to an action of the peptide to inhibit water drinking.

The obestatin concerning study for energy homeostasis and body weight regulation could be expected to have a large development in the future. The Human Obestatin ELISA developed by our laboratory can be used for direct determination of blood obestatin level variations and will be a useful tool for further development of obestatin research.

PRINCIPLE

This ELISA kit is used for quantitative determination of obestatin in human serum and plasma samples. It has various advantages, such as highly specific and sensitive quantification, no influences with other body fluids or physiological active substances and unnecessity of sample pretreatment. The human obestatin calibrator of this kit is a highly purified synthetic product (purity: higher than 99%).

The ELISA kit shows cross-reactivity of 100% to human obestatin, 37.3% to mouse/rat obestatin, 25.2% to human obestatin (11-23)-NH_2, less than 0.02% to human/mouse/rat obestatin (1-10), and no cross-reactivity to mouse/rat obestatin (11-23)-NH_2. It shows no cross-reactivity to human ghrelin and human des-octanoyl ghrelin in the range of the calibrator concentrations.

This ELISA kit for determination of obestatin in human serum and plasma samples is based on a competitive enzyme immunoassay using the combination of highly specific antibody to human obestatin and biotin–avidin affinity system. The 96 wells plate is coated with goat anti-rabbit IgG, to which biotinylated human obestatin, human obestatin calibrator or samples and rabbit anti-human obestatin antibody are added for competitive immunoreaction. After incubation and plate washing, horseradish peroxidase (HRP) labeled streptavidin (SA) is added, so that HRP labeled SA-biotinylated human obestatin-antibody complex is formed on the surface of the wells. Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of human obestatin is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	Microtiter plate	1 plate (96-well)	Goat anti-rabbit IgG
2. Calibrator	Lyophilized	1 vial (25 ng)	Synthetic human obestatin
3. Labeled Antigen	Lyophilized	1 vial	Biotinylated human obestatin
4. Specific Antibody	Liquid	1 bottle (6 mL)	Rabbit anti-human obestatin antibody
5. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-labeled streptavidin
6. TMB Substrate	Liquid	1 bottle (12 mL)	TMB (3,3',5,5'-tetramethylbenzidine)
7. Stop Solution	Liquid	1 bottle (12 mL)	1 M H_2SO_4
8. Buffer Solution	Liquid	1 bottle (25 mL)	BSA containing saline buffer
9. Wash Solution Concentrate	Liquid	1 bottle (25 mL)	Concentrated saline
10. Plate Seal		1 sheets	

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 450 nm
- Washing device for microtiter plate and dispenser with aspiration system
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips (20 µL - 1 mL)
- Test tubes (glass or polypropylene) for preparation of calibrator solution
- Graduated cylinder (500 mL or 1,000 mL)
- Distilled or de-ionized water
- Lint free paper towel
- A microplate shaker if necessary

PRECAUTIONS

Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

Satisfactory performance of the test is guaranteed only when reagents are used from a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette the prepared Calibrator Solutions or samples into corresponding wells. Use a new tip for each sample and calibrator solution to avoid cross-contamination. Use clean test tubes or vessels.

Always run a calibration curve when testing samples.

Calibrator and labeled antigen solutions should be prepared immediately before use. The plate can be used twice separately. In that case, the rest of the reconstituted calibrator and labeled antigen solution should be stored below -30°C but others at 4℃ and used in 2 weeks.

Color reaction should be carried out in light-proof condition.

It is strongly recommended protease inhibitors (e.g. pepstatin A 10 µM or inhibitors cocktail) should be added to serum or plasma samples immediately after separation and kept in an ice-bath until assay. A slight arising of determination value may be observed after addition of pepstatin A. This value arising phenomena are not ineligible especially when use of cocktail inhibitor in its effective concentrations. Aprotinin was observed not to change determination value, but not effectively to inhibit protease up to addition of 0.8 TIU/mL in serum tested in our recent experiment. If the sample is tested later, they should be divided aliquoted and frozen below -30℃ (for long term storage, stored in a -80℃ deep freezer). During thawing of sample before assay, it should be kept in an ice-bath and used as soon as possible. Repeated freezing and thawing of samples should be avoided.

Perform all the determinations in duplicate or more.

TMB Substrate solution should be equilibrated at least 1 hour at room condition to room temperature before applying. It is supposed that low or high temperature of TMB substrate solution which if added to plate may affect the color levels remarkably.

Read optical densities of reaction solution in wells immediately after the reaction stopping.

If multiple assay kits will be used, please run all assay kits always on consistent conditions (e.g. incubation time, temperature, shake speed etc.) to get optimal inter-assay performance.

REAGENT PREPARATION

1. Preparation of Calibrator Solution: Reconstitute the Calibrator (lyophilized human obestatin 25 ng/vial) with 1 mL of Buffer Solution, which affords 25 ng/mL calibrator solution. The reconstituted calibrator solution (0.1 mL) is diluted with 0.2 mL of Buffer Solution that yields 8.333 ng/mL calibrator solution. Repeat the same dilution to make calibrator solutions of 2.778, 0.926 ng/mL. Dilute the calibrator solution (0.926 ng/mL) 0.1 mL with 0.1 mL of Buffer Solution that yields 0.463 ng/mL calibrator solution. Repeat dilution of the calibrator solution (0.426 ng/mL) 0.1 mL with 0.1 mL of Buffer Solution to yield 0.231 ng/mL calibrator solution. Buffer Solution is used as 0 ng/mL.

2. Preparation of labeled antigen: Reconstitute labeled antigen with 6 mL of buffer solution.

3. Preparation of Wash Solution: Dilute 25 mL of Wash Solution Concentrate to 500 mL with distilled or de-ionized water.

4. The other reagents are ready for use.

STORAGE

Store kit at 4°C.

ASSAY PROTOCOL

Note: Before starting assay, bring all the reagents except samples to room temperature (20-25°C). Samples should be kept in an ice-bath after separation or during thawing from freezing and preferably be used in as soon as possible.

1. Add 300 µL of washing solution to each well and keep it for at least 30 seconds, then aspirate or decant the washing solution in the wells. **Invert the plate and tap it firmly on a lint free paper towel to ensure blotting free of most residual washing solution.**

2. Fill 50 µL of labeled antigen solution into each well first, then introduce 20 µL of each of calibrator solutions (0, 0.231, 0.463, 0.926, 2.778, 8.333, 25 ng/mL) or samples and finally add 50 µL of human obestatin antibody solution into each well.

3. Cover the plate with Adhesive Foil and incubate it at 4°C for 20 - 22 hours (still).

4. After incubation, take off the Adhesive Foil, aspirate the contents, then add 300 µL of washing solution to each well and aspirate. Repeat the wash step for total of five times with approximately 300 µL/well of washing solution each time and **finally invert the plate and tap it firmly on a lint free paper towel to ensure blotting free of most residual washing solution.**

5. Pipette 100 µL of SA-HRP Solution into each well.

6. Cover the plate with Adhesive Foil and incubate it at room temperature for 1 hour with shaking.

7. Take off the Adhesive Foil, aspirate and wash the wells five times as in step 4.

8. Add 100 µL of TMB Substrate into each well; cover the plate with Adhesive Foil and keep it for 30 minutes with shaking at room temperature under a light proof condition.

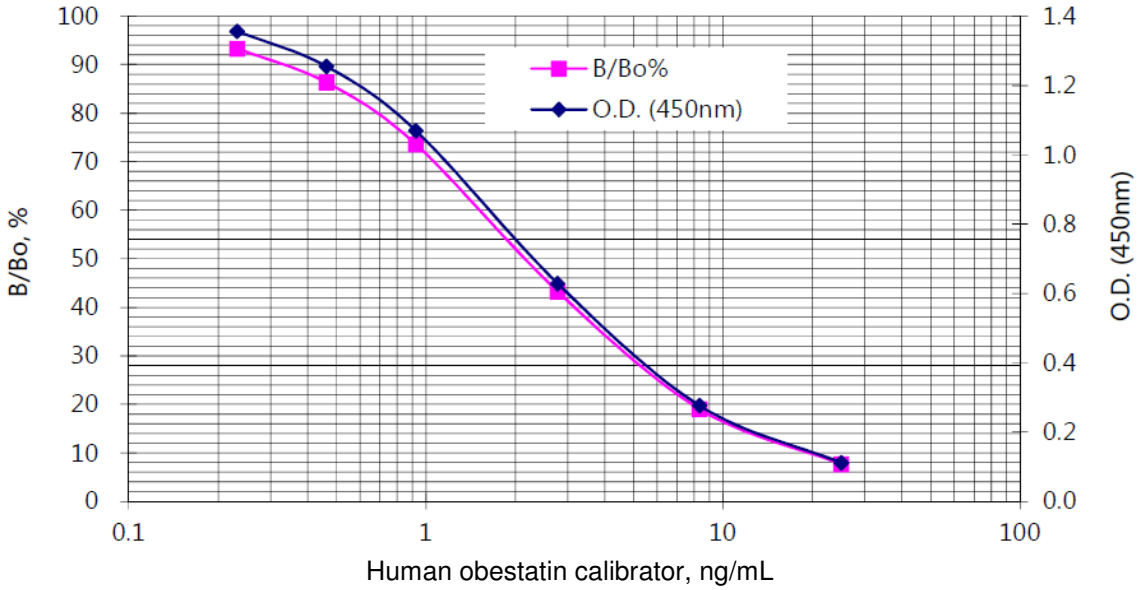
9. Add 100 µL of Reaction Stopping Solution into each well to stop coloring reaction.

10. Read the optical absorbance of the wells at 450 nm.

RESULTS

Calculate mean optical density values of wells containing calibrator solutions or their bound percentage (B/Bo%) to Bo wells (0 ng/mL calibrator as Bo) and plot a calibration curve on a semi-logarithmic graph paper (abscissa: concentrations of calibrator; ordinate: optical density or B/Bo%). Use the average optical density or B/Bo% of each sample to determine the corresponding value by simple interpolation from the calibration curve.

An example of human obestatin ELISA calibration curve



Precision and Reproducibility

- Intra-assay CV (%) 3.5 – 9.9
- Inter-assay CV (%) 5.6 – 9.0

Assay Range

0.231 – 25 ng/mL

Sensitivity

$(2 \times SD_{0\ ng/mL} \times 0.231\ ng/mL)/(O.D._{0\ ng/mL} - O.D._{0.231\ ng/mL})$

Analytical Recovery

Human Serum	101.5 – 113.2% (n=7)
Human Plasma	106.1 – 118.9% (n=7)

Dilution Test

Linear dilution characteristics were shown with human serum and human plasma at least up to 8 fold and 4 fold respectively.

FOR RESEARCH USE ONLY

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