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.

PRODUCT INFORMATION

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INTENDED USE
The Human Obestatin ELISA is for the quantitative determination of obestatin in human serum and plasma. For research use only.

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 (50 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. aprotinin or inhibitors cocktail) should be added to serum or plasma samples immediately after separation and kept in an ice-bath until assay. 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. It might be suggested as a rational method adding some content of protease inhibitors to the kit Buffer Solution before preparation of calibrators and labeled antigen solution for obtaining optimal reproductive results if not so satisfactory reproductivity is encountered with sample determinations. 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 Human Obestatin Calibrator with 0.5 mL of buffer solution, which makes a 100 ng/mL Calibrator Solution. Add 0.1 mL of the 100 ng/mL Calibrator Solution with 0.2 mL of Buffer Solution, which yields a 33.333 ng/mL Calibrator Solution. Repeat the same dilution procedure to make 11.111, 3.704, 1.235 and 0.412 ng/mL Calibrator Solutions. Buffer Solution is used as the 0 ng/mL Calibrator.

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

Before starting assay, bring all the reagents except samples to room temperature (20-25°C). Protease inhibitor added serum and plasma 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 0.30 mL/well of diluted Wash Solution into the wells of the plate and keep it for about 30 seconds, then aspirate the solution. Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash Solution.
2. Add 50 µL of labeled antigen solution, and then add 20 µL of each prepared Calibrator Solution (0, 0.412, 1.235, 3.704, 11.111, 33.333 and 100 ng/mL) or samples and finally add 50 µL of human obestatin antibody into the wells.
3. Cover the plate with a Plate Seal and incubate at 4°C overnight for 18 – 20 hours and then incubate further 30 minutes at room temperature. (Still or shaking.)
4. After incubation, remove the Plate Seal, aspirate the solution in the wells and wash the wells 5 times as before with approximately 0.30 mL/well of diluted Wash Solution. Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash Solution.
5. Pipette 100 µL of SA-HRP Solution into each of the wells.
6. Cover the plate with a Plate Seal and incubate at room temperature for 1 hour. (Still or shaking).
7. Remove the Plate Seal and aspirate the solution in the wells and then wash the wells 5 times as before with approximately 0.30 mL/well of diluted Washing Solution.
8. Add 100 µL of TMB solution into each of the wells, cover the plate with a Plate Seal and incubate for 30 minutes at room temperature, protected from light. (Still or shaking).
9. Add 100 µL of Stop Solution into each of the wells.
10. Read optical absorbance of the solution in 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.

Precision and Reproducibility

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

Assay Range

0.412 – 100 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.

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