KAMIYA BIOMEDICAL COMPANY

Mouse and Rat Obestatin ELISA

For the quantitative determination of obestatin in mouse and rat serum

Cat. No. KT-494

For Research Use Only.

PRODUCT INFORMATION

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INTENDED USE
The Mouse and Rat Obestatin ELISA is for the quantitative determination of obestatin in mouse and rat serum. 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 Mouse and Rat Obestatin ELISA developed by our laboratory can be used for direct determination of serum 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 mouse/rat serum 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 mouse/rat obestatin calibrator of this kit is a highly purified synthetic product (purity: higher than 99%).

The ELISA kit shows cross-reactivity of 100% to mouse/rat obestatin, 118.6% to mouse/rat obestatin (11-23)-NH2, 0.5% to mouse/rat obestatin (1-23)-OH and less than 0.39% to human/mouse/rat obestatin (1-10) and no cross-reactivity to human obestatin, human obestatin (11-23))-NH2, and mouse/rat ghrelin and mouse/rat des-octanoyl ghrelin.

This ELISA kit for determination of obestatin in mouse/rat serum samples is based on a competitive enzyme immunoassay using the combination of highly specific antibody to mouse/rat obestatin and biotin–avidin affinity system. The 96 wells plate is coated with goat anti-rabbit IgG, to which biotinylated mouse/rat obestatin, mouse/rat obestatin calibrator or samples and rabbit anti-mouse/rat 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 mouse/rat 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 mouse/rat 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 (20 ng)	Synthetic mouse/rat obestatin
3. Labeled Antigen	Lyophilized	1 vial	Biotinylated mouse/rat obestatin
4. Specific Antibody	Liquid	1 bottle (6 mL)	Rabbit anti-mouse/rat 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		3 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
- Test tubes (glass or polypropylene) for preparation of calibrator solution
- Graduated cylinder (500 mL or 1,000 mL)
- Distilled water or de-ionized water

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.

Reconstituted reagents should be stored below -30°C if not used all at once.

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

Aprotinin (0.6TIU/mL) must be added to serum samples as soon as possible after separation. If the sample is tested later, they should be divided into test tubes in small amounts and frozen below –30℃ (for long term storage, it is recommended the sample should be stored in a –80℃ deep freezer). Avoid repeated freezing and thawing of samples. During thawing of frozen samples before assay, they should be kept in an ice bath and used within 60 minutes.

REAGENT PREPARATION

1. Preparation of Calibrator Solution: Reconstitute the Mouse/Rat Obestatin Calibrator with 1 mL of buffer solution, which makes a 20 ng/mL Calibrator Solution. Add 0.1 mL of the 20 ng/mL Calibrator Solution with 0.2 mL of Buffer Solution, which yields a 6.667 ng/mL Calibrator Solution. Repeat the same dilution procedure to make 2.222, 0.741, 0.247 and 0.082 ng/mL Calibrator Solutions. Buffer Solution is used as the 0 ng/mL Calibrator.

   Note: Calibrator Solution should be prepared immediately before use.

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

   Note: Labeled Antigen Solution should be prepared immediately before use.

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

Note: During storage of Wash Solution Concentrate at 4°C, precipitates may be observed. However, they will be dissolved when diluted. Diluted Wash Solution is stable for 6 months at 4°C.

4. The other reagents are ready for use.

STORAGE

Store kit at 4°C.

ASSAY PROTOCOL

1. Bring all the reagents except samples to room temperature (20-30°C) before starting assay.
2. Add 0.35 mL/well of diluted Wash Solution into the wells of the plate and keep it for about 30 seconds, then aspirate the solution. Repeat this washing procedure twice (total 3 times). Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash Solution.
3. Add 50 µL of labeled antigen solution, and then add 25 µL of each prepared Calibrator Solution (0, 0.082, 0.247, 0.741, 2.222, 6.667 and 20 ng/mL) or samples and finally add 50 µL of mouse/rat obestatin antibody into the wells.
4. Cover the plate with a Plate Seal and incubate at 4°C overnight for 18 – 20 hours. (Still, plate shaker not needed.)
5. After 4°C incubation, remove the Plate Seal, aspirate the solution in the wells and wash the wells 3 times as before with approximately 0.35 mL/well of diluted Wash Solution.
6. Pipette 100 µL of SA-HRP Solution into each of the wells.
7. Cover the plate with a Plate Seal and incubate at room temperature for 1 hour. (Still, plate shaker not needed.)
8. Remove the Plate Seal and aspirate the solution in the wells and then wash the wells 5 times as before with approximately 0.35 mL/well of diluted Washing Solution.
9. 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, plate shaker not needed.)
10. Add 100 µL of Stop Solution into each of the wells.
11. Read optical absorbance of the solution in the wells at 450 nm. Read plate optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

Notes: Perform all determinations in duplicate.

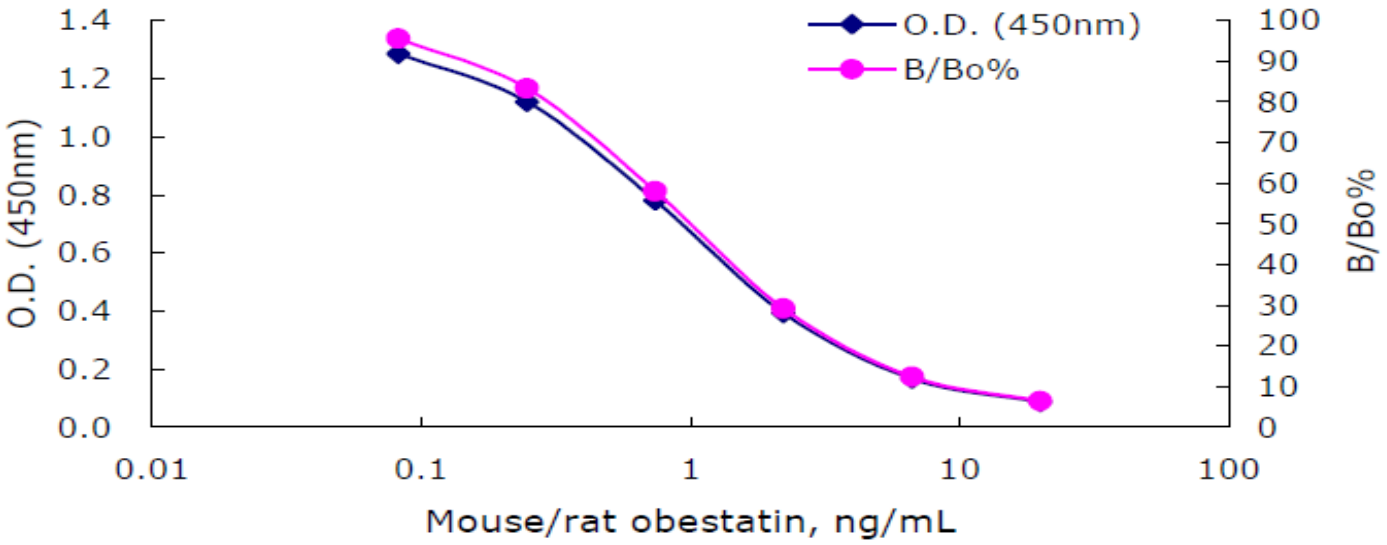
RESULTS

Calculate mean absorbance values of calibrators and plot a calibration curve on semi-logarithmic graph paper (abscissa: concentration of calibrator; ordinate: absorbance values).

Use the calibration curve to read mouse/rat obestatin concentrations in samples from the corresponding absorbance values.

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Precision and Reproducibility

•	Intra-assay CV (%)	Mouse Serum	3.7 – 6.9
		Rat Serum	3.4 – 6.7
•	Inter-assay CV (%)	Mouse Serum	4.5 – 8.4
		Rat Serum	8.1 – 10.8

Assay Range
0.082 – 20 ng/mL

Analytical Recovery

Mouse Serum	102.7 – 108.9% (n=4)
Rat Serum	85.7 – 95.7% (n=3)

Dilution Test
Satisfactory dilution characteristics were shown with mouse and rat serum.

FOR RESEARCH USE ONLY

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