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Research
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# Human Obestatin ELISA

Cat. No.: RSCYK231R

## 1. INTRODUCTION

Obestatin is a 23 amino acid residues peptide isolated from the 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 [(1)]. 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 [(2)]. Obestatin (100nM) added to dissociated and cultured rat cerebral cortical neurons elevated cytosolic calcium concentrations [Ca+2]i in a population of cortical neurons [(2)]. Intracerebroventricular admini- stration 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 [(3)]. Obestatin peptides had no effect on insulin sensitivity as revealed by hypoglycaemic 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 [(4)]. 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 [(3)].

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 EIA assay kit developed by our laboratory can be used for direct determination of blood obestatin level's variations and will be a useful tool for further development of obestatin research.

| | RSCYK231R Human Obestatin EIA Kit | | Contents |
|---|---|---|---|
| ▼ | The assay kit can measure human obestatin within the range of 0.412-100 ng/mL. | 1) | Antibody coated plate |
| ▼ | The assay completes within 18-20 h. + 1.5 h. | 2) | Standard |
| ▼ | With one assay kit, 41 samples can be measured in duplicate. | 3) | Labeled antigen |
| ▼ | Test sample: human plasma or serum. Sample volume: 20 µL. | 4) | Specific antibody |
| ▼ | The 96-well plate of this kit is consists of 8-well strips, so that divided use by strips is possible at user's option. | 5) | SA-HRP solution |
| ▼ | Intra-assay CV（%）3.5-9.9. | 6) | TMB substrate |
| ▼ | Inter-assay CV（%）5.6-9.0. | 7) | Reaction stopping solution |
| | | 8) | Buffer solution |
| | Store all the components at 2-8℃. The kit is stable under the condition for 6 months from the date of manufacturing. | 9) | Washing solution (concentrated) |
| | The expiry date is stated on the package. | 10) | Adhesive foil |

## 2. CHARACTERISTICS

This EIA kit is used for quantitative determination of obestatin in human plasma and 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. Human obestatin standard of this kit is a highly purified synthetic product (purity: higher than 99%).

**Specificity**
The EIA 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 standard concentrations.

**Assay Principle**

This EIA kit for determination of obestatin in human plasma or serum 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 standard or samples and rabbit anti human obestatin antibody are added for competitive immunoreaction. After incubation and plate washing, horse radish 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.

## 3. COMPOSITION

| | Component | Form | Quantity | Main Ingredient |
|---|---|---|---|---|
| 1. | Antibody coated plate | Microtiter plate | 1 Plate (96 wells) | Goat anti rabbit IgG |
| 2. | Standard | Lyophilized powder | 1 Vial (50ng) | Synthetic human obestatin |
| 3. | Labeled antigen | Lyophilized powder | 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) | 3,3',5,5'-Tetramethylbenzidine (TMB) |
| 7. | Reaction Stopping solution | Liquid | 1 Bottle (12 mL) | 1M $H_2SO_4$ |
| 8. | Buffer solution | Liquid | 1 Bottle (25 mL) | BSA containing saline buffer |
| 9. | Washing solution (concentrated) | Liquid | 1 Bottle (25 mL) | Concentrated saline |
| 10 | Adhesive foil | | 3 Sheets | |

# 4. METHOD

## Equipment required

1. Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450 nm
2. Washing device for microtiter plate and dispenser with aspiration system
3. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
4. Test tubes (glass or polypropylene) for preparation of standard solution
5. Graduated cylinder (500 mL or 1,000 mL)
6. Distilled or deionized water

## Preparatory work

1. Preparation of standard solution:
   Reconstitute the human obestatin standard (lyophilized human obestatin 50 ng/vial) with 0.5 mL of buffer solution, which affords 100 ng/mL standard solution. The reconstituted standard solution (0.1mL) is diluted with 0.2 mL of buffer solution that yields 33.333 ng/mL standard solution. Repeat the same dilution to make each standard solution of 11.111, 3.704, 1.235, and 0.412 ng/mL. Buffer solution is used as 0 ng/mL.
2. Preparation of labeled antigen solution:
   Reconstitute labeled antigen with 6 mL of buffer solution.
3. Preparation of washing solution:
   Dilute 25 mL of washing solution (concentrated) to 500 mL with distilled or deionized water.
4. Other reagents are ready for use.

## Procedure

1. Before starting assay, bring all the reagents except samples to room temperature (20-30°C).
2. Add 350 µL of washing solution to each well and keep it for about 30 seconds, then aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
3. Fill 50 µL of labeled antigen solution into each well first, then introduce 20 µL of each of standard solutions (0, 0.412, 1.235, 3.704, 11.111, 33.333, 100 ng/mL) or samples and finally add 50 µL of human obestatin antibody solution into each well.

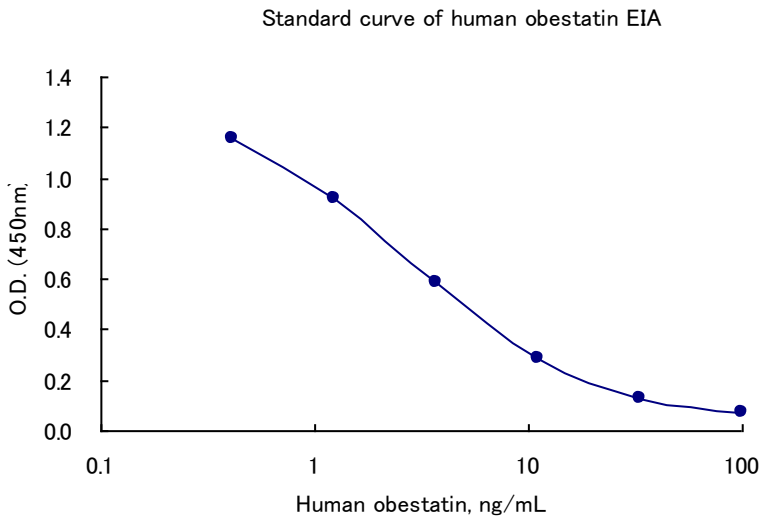
4. Cover the plate with adhesive foil and incubate it at 4°C for 18 - 20 hours and further more 30 minutes at room temperature (still, no shaking).
5. After incubation, take off the adhesive foil, aspirate and wash the wells three times as step 2 with approximately 0.35mL/well of washing solution each time.
6. Pipette 100 µL of SA-HRP solution into each well.
7. Cover the plate with adhesive foil and incubate it at room temperature for 1 hour (still, no shaking).
8. Take off the adhesive foil, aspirate and wash the wells five times as step 2 with approximately 0.35 mL/well of washing solution each time.
9. Add 100 µL of TMB solution into each well; cover the plate with adhesive foil and keep it for 30 minutes at room temperature under a light proof condition (still, no shaking).
10. Add 100 µL of reaction stopping solution into each well to stop color reaction.
11. Read the optical absorbance of the wells at 450 nm.
12. Calculate mean absorbance values of standards and plot a standard curve on semi-logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the standard curve to read human obestatin concentrations in samples from the corresponding absorbance values.

## 5. NOTES

1. It is recommended aprotinin (0.6 TIU/mL) should be added to serum or plasma samples as soon as possible after separation. If the sample is tested later, they should be divided aliquoted and frozen below – 30°C (for long term storage, it is recommended the sample be stored in a – 80°C 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.
2. Standard and labeled antigen solutions should be prepared immediately before use. The plate can be used for separately twice. In that case, the rests of the reconstituted reagents (standard and labeled antigen solution) should be stored below -30°C.
3. During storage of washing solution (concentrated) at 2 - 8°C, precipitates may be observed occasionally, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2 - 8°C.
4. As pipetting operations may affect precision of the assay, pipette standard solutions or samples precisely into each well of plate. In addition, use clean test tubes or vessels in assay and use a new tip for each sample or standard solution and for each standard diluting process to avoid cross contamination.
5. Perform all the determination in duplicate.

6. To quantitate accurately, always run a standard curve when testing samples.
7. Color reaction should be carried out under the light proof condition.
8. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.
9. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
10. Satisfactory performance of the assay will be guaranteed only when reagents are used from combination pack with identical lot number.

## 6. PERFORMANCE CHARACTERISTICS




**Precision and reproducibility**
Intra-assay CV(%): 3.5 ~ 9.9
Inter-assay CV(%): 5.6 ~ 9.0

**Assay range**
0.412 – 100ng/mL

**Analytical recovery**
Human serum: 101.5~113.2% (n=7)
Human plasma: 106.1~118.9% (n=7)

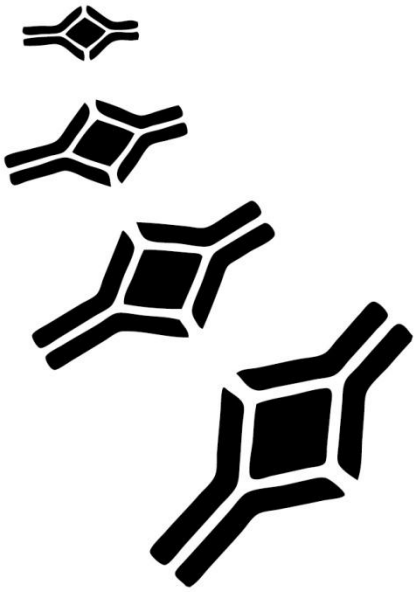
**Dilution test**
Linear dilution characteristics were shown with human serum and human plasma at least up to 8 folds and 4 folds respectively.

## 7. STABILITY AND STORAGE

**Storage** Store all the components at 2 to 8°C.
**Shelf life** Kit is stable under the condition for 6 months from the date of manufacturing.The expiry date is stated on the label of package.
**Package** For 96 tests per one kit including standards.

## 8. REFERENCES


1. Zhang JV, Ren PG et al: Obestatin, a peptide encoded by the ghrelin gene, opposes ghrelin's effects on food intake. Science 310:996-999, 2005
2. Dun SL, Brailoiu GC et al: Distribution and biological activity of obestatin in the rat. J Endocrinol 191:1–10, 2006
3. Samson WK, White MM et al: Obestatin acts in brain to inhibit thirst. Am J Physol: Regulatory, Integrative and Comparative Physiolgy 292 (1): R637-643, 2007; Epub 2006 Aug 24
4. Green BD, Irwin N and Flatt PR: Direct and indirect effects of obestatin peptides on food intake and the regulation of glucose homeostasis and insulin secretion in mice. Peptides 28:981-987, 2007; Epub 2007 Feb 12



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