

YK230 Mouse/Rat Obestatin EIA

FOR LABORATORY USE ONLY

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– Please read all the package insert carefully before beginning the assay –

YK230 Mouse/Rat Obestatin EIA

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

YK230 Mouse/rat Obestatin EIA Kit	Contents
The assay kit can measure mouse/rat obestatin within the range of 0.082~20ng/mL.	1) Antibody coated plate
The assay completes within 18~20h. + 1.5 h.	2) Standard
With one assay kit, 41 samples can be measured in duplicate.	3) Labeled antigen
Test sample: mouse or rat serum.	4) Specific antibody
Sample volume: 25 μ L.	5) SA-HRP solution
The 96-well plate of this kit is consists of 8-well strips, The kit can be used separately.	6) TMB substrate
Intra-assay CV(%) 3.7~6.9 (mouse serum), 3.4~6.7 (rat serum).	7) Reaction stopping solution
Inter-assay CV(%) 4.5~8.4 (mouse serum), 8.1~10.8 (rat serum).	8) Buffer solution
Store all the components at 2~8 . The kit is stable under the condition for 24 months from the date of manufacturing.	9) Washing solution (concentrated)
The expiry date is stated on the package.	10) Adhesive foil

. Characteristics

This EIA 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 unnecessary of sample pretreatment. Mouse/rat 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 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, mouse/rat ghrelin and mouse/rat des-octanoyl ghrelin.

< Assay Principle >

This EIA 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 standard or samples and rabbit anti mouse/rat 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 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.

. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	Microtiter plate	1 plate (96 wells)	Goat anti rabbit IgG
2. Standard	Lyophilized	1 vial (20ng)	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)	3,3',5,5'-Tetramethylbenzidine (TMB)
7. Reaction Stopping solution	Liquid	1 bottle (12 mL)	1M H ₂ SO 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	

. Method

< Equipment required >

1. Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450nm
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 (500mL)
6. Distilled or deionized water

< Preparatory work >

1. Preparation of standard solution:
Reconstitute the mouse/rat obestatin standard (lyophilized mouse/rat obestatin 20ng/vial) with 1mL of buffer solution, which affords 20ng/mL standard solution. The reconstituted standard solution (0.1mL) is diluted with 0.2mL of buffer solution that yields 6.667ng/mL standard solution. Repeat the same dilution to make each standard solution of 2.222, 0.741, 0.247, and 0.082ng/mL. Buffer solution is used as 0ng/mL.
2. Preparation of labeled antigen solution:
Reconstitute labeled antigen with 6mL of buffer solution.
3. Preparation of washing solution:
Dilute 25mL of washing solution (concentrated) to 500mL with distilled or deionized water.
4. Other reagents are ready for use.

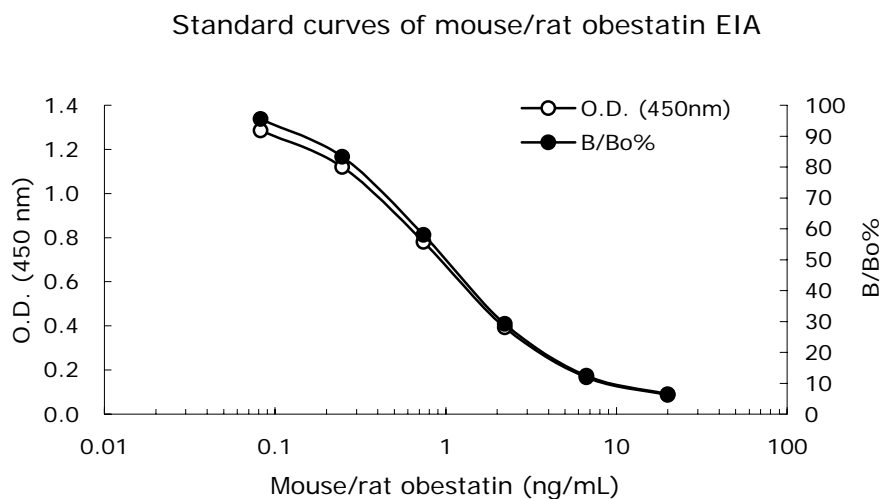
< Procedure >

1. Before starting assay, bring all the reagents except samples to room temperature (20-30°C).
2. Add 350µL 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 50µL of labeled antigen solution into each well first, then introduce 25µL of each of standard solutions (0, 0.082, 0.247, 0.741, 2.222, 6.667, 20ng/mL) or samples and finally add 50µL of specific antibody into each well.
4. Cover the plate with adhesive foil and incubate it at 4°C for 18-20 hours (still, no shaking).
5. After 4°C 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 100µL 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 0.35mL/well of washing solution each time.
9. Add 100µL of TMB solution into each well, cover the plate with adhesive foil and keep it for 30 minutes at room temperature under a lightproof condition (still, no shaking).
10. Add 100µL of reaction stopping solution into each well to stop color reaction.
11. Read the optical absorbance of the wells at 450nm.
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 mouse/rat obestatin concentrations in samples from the corresponding absorbance values.

. Notes

1. Aprotinin (0.6TIU/mL) must be added to serum sample as soon as possible after separation. If the sample is tested later, they should be divided into test tubes in small amount and frozen below -30°C (for long term storage, it is recommended the sample should 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 $2-8^{\circ}\text{C}$, precipitates may be observed occasionally, however they will be dissolved when diluted.
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 pipetting each standard diluting process, sample or standard solution 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 in the lightproof 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.

. Performance Characteristics



< 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 >

0.082~20ng/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.

. Stability and Storage

- < **Storage** > Store all the components at 2~8°C.
- < **Shelf life** > The kit is stable under the condition for 24 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.

. 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 Physiol: Regulatory, Integrative and Comparative Physiology 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

< **Manufacturer** >

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