
**YK230 Mouse/Rat Obestatin EIA
Product Instruction**

FOR LABORATORY USE ONLY

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

YK230 Mouse/Rat Obestatin EIA

I . 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 [Ca²⁺]_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⁾. Recently, it is reported affording cardioprotection to ischemic-reperfused isolated rat heart, inhibiting apoptosis in culture of similarly stressed cardiomyocytes⁽⁵⁾ and inhibiting dopamine release in rat hypothalamus⁽⁶⁾.

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. Sample volume: 25 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 (%) 3.7 ~ 6.9 (mouse serum), 3.4~6.7 (rat serum).	6)TMB Substrate
▼Inter-assay CV (%) 4.5 ~ 8.4 (mouse serum), 8.1~10.8 (rat serum).	7)Reaction Stopping Solution
Store all the components at 2-8°C.	8)Buffer Solution
The expiry date is stated on the package.	9)Concentrated Wash Solution
	10)Adhesive Foil

II. 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, and 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.

III. 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 (20ng)	Synthetic mouse/rat obestatin
3	Labeled Antigen	lyophilized powder	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	Concentrated Wash Solution	liquid	1 bottle (50 mL)	Concentrated saline
10	Adhesive Foil		3 sheets	

IV. 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 or 1,000mL)
6. Distilled or deionized water
7. Lint free paper towel
8. A microplate shaker if necessary

< 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 50mL of Concentrated Wash Solution to 1000mL with distilled or deionized water.
4. Other reagents are ready for use.

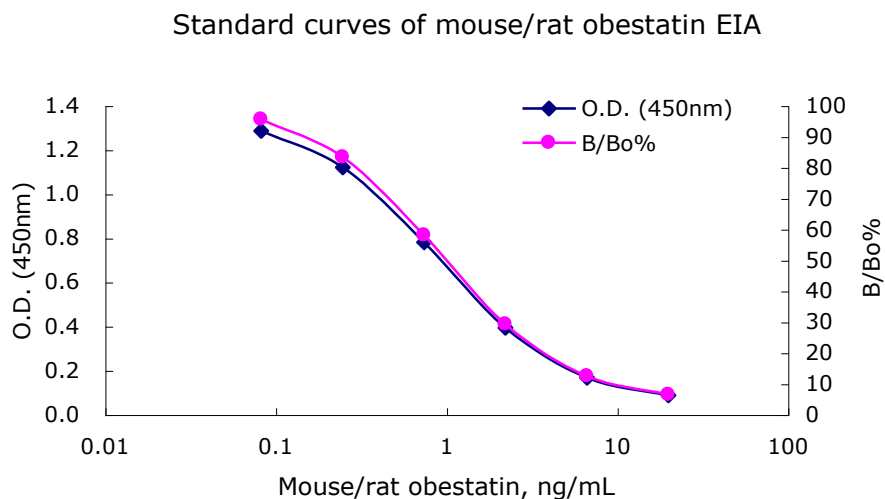
< Procedure >

1. Before starting assay, bring all the reagents **except samples** to room temperature (20-25°C). The sample should be kept in ice-bath after thawing.
2. Add 350µL 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.**
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 mouse/rat obestatin 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 incubation, take off the Adhesive Foil, aspirate the contents, then add 350µL of washing solution to each well and aspirate. Repeat the wash step for total of five times with approximately 350µL/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.**
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 **Procedure 5.**
9. Add 100µL of TMB Substrate into each well, cover the plate with Adhesive Foil and keep it for 30 minutes (still, no shaking) at room temperature under a light proof condition (please refer to **V. Notes 7.** for more information).
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 optical density values of wells containing standard solutions or their bound percentage (B/Bo%) to Bo wells (0 ng/mL standard as Bo) and plot a standard curve on a semi-logarithmic graph paper (abscissa: concentrations of standard; 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 standard curve.

V. Notes

1. It is strongly recommended that protease inhibitors (e.g. aprotinin or some cocktail 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 twice separately. In that case, the rests of the reconstituted standard and labeled antigen solution should be stored below -30°C but others at 4°C and used in 2 weeks.
3. As pipetting operations may affect precision of the assay, pipette standard solutions or samples precisely into each well of plate. In addition, use a new tip for each sample or standard solution and for each standard diluting process to avoid cross contamination.
4. Perform all the determination in duplicate or more.
5. Always make a standard curve when testing samples because the assay conditions may be different to each other that influence the coloring levels and result precisions.
6. Coloring reaction should be carried out under the light proof condition.
7. 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.
8. Read optical densities of reaction solution in wells immediately after the reaction stopping.
9. 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.
10. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
11. Satisfactory performance of the assay will be guaranteed only when reagents are used from combination pack with identical lot number.

VI. 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 and Sensitivity >

Range: 0.082 – 20 ng/mL;

Sensitivity = $(2 \times SD_{0\text{ng/mL}} \times 0.082\text{ng/mL}) / (O.D._{0\text{ng/mL}} - O.D._{0.082\text{ng/mL}})$

< 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.

VII. Stability and Storage

- < Storage >** Store all the components at 2 to 8°C.
- < Shelf life >** Kit is stable under the condition for 24 months from the manufacturing date.
The expiry date is stated on the label of package.
- < Package >** For 96 tests per one kit including standards.

VIII. 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
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
5. Alloatti G, Arnoletti E et al: **Obestatin affords cardioprotection to the ischemic-reperfused isolated rat heart and inhibits apoptosis in cultures of similarly stressed cardiomyocytes.** Am J Physiol Heart Circ Physiol 299 (2):H470-81, 2010
6. Brunetti L, Di Nisio C et al: **Obestatin inhibits dopamine release in rat hypothalamus.** Eur J Pharmacol 641 (2-3):142-7, 2010

< Manufacturer >

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