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**YK231 Human Obestatin EIA  
Product Instructions**

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**FOR LABORATORY USE ONLY**

**YANAIHARA INSTITUTE INC.  
2480-1 AWAKURA, FUJINOMIYA-SHI  
SHIZUOKA, JAPAN 418-0011**

Our website: [www.yanaihara.co.jp](http://www.yanaihara.co.jp) E-mail: [ask@yanaihara.co.jp](mailto:ask@yanaihara.co.jp)

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

## YK231 Human 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 <sup>(1)</sup>. 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 <sup>(2)</sup>. Obestatin (100nM) added to dissociated and cultured rat cerebral cortical neurons elevated cytosolic calcium concentrations [Ca<sup>2+</sup>]<sub>i</sub> in a population of cortical neurons <sup>(2)</sup>. 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 <sup>(3)</sup>. 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 <sup>(4)</sup>. 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 <sup>(3)</sup>. Recently, it is reported affording cardioprotection to ischemic-reperfused isolated rat heart, inhibiting apoptosis in culture of similarly stressed cardiomyocytes<sup>(5)</sup> and inhibiting dopamine release in rat hypothalamus<sup>(6)</sup>.

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.

YK231 Human Obestatin EIA Kit	Contents
The kit assay range: 0.231-25ng/mL.	1)Antibody Coated Plate
The assay completes within 20-22 h. + 1.5 h.	2)Standard
41 Samples can be measured in duplicate.	3)Labeled Antigen
Test sample: human plasma or serum. Sample volume: 20 µL.	4)Specific Antibody
8-Well strips consisted plate is possible for divided use.	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 .	9)Concentrated Wash Solution
The expiry date is stated on the package.	10)Adhesive Foil

## II. 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 unnecessary 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<sub>2</sub>, less than 0.02% to human/mouse/rat obestatin (1-10), and no cross-reactivity to mouse/rat obestatin (11-23)-NH<sub>2</sub>. 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.

### . 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 (25ng)	Synthetic human obestatin
3.	Labeled Antigen	Lyophilized powder	1 Vial	Biotinylated human obestatin
4.	Specific Antibody	Liquid	1 Bottle (6mL)	Rabbit anti human obestatin antibody
5.	SA-HRP Solution	Liquid	1 Bottle (12mL)	HRP labeled streptavidin
6.	TMB Substrate	Liquid	1 Bottle (12mL)	3,3',5,5'-Tetramethylbenzidine (TMB)
7.	Reaction Stopping Solution	Liquid	1 Bottle (12mL)	1M H <sub>2</sub> SO <sub>4</sub>
8.	Buffer Solution	Liquid	1 Bottle (25mL)	BSA containing saline buffer
9.	Concentrated Wash Solution	Liquid	1 Bottle (25mL)	Concentrated saline
10	Adhesive Foil		1 Sheet	

#### IV. Method

**Note: Before starting assay, bring all the reagents except samples to room temperature (20-25°C). Samples should be kept in an ice-bath after separation or during thawing from freezing and preferably be used in as soon as possible. Please refer to V. Notes 1. for detail.**

< 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 (20 $\mu$ L-1mL)
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 Standard (lyophilized human obestatin 25ng/vial) with 1mL of Buffer Solution, which affords 25ng/mL standard solution. The reconstituted standard solution (0.1mL) is diluted with 0.2mL of Buffer Solution that yields 8.333ng/mL standard solution. Repeat the same dilution to make standard solutions of 2.778, 0.926ng/mL. Dilute the standard solution (0.926ng/mL) 0.1mL with 0.1mL of Buffer Solution that yields 0.463ng/mL standard solution. Repeat dilution of the standard solution (0.426ng/mL) 0.1mL with 0.1mL of Buffer Solution to yield 0.231ng/mL standard solution. 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 Concentrated Wash Solution to 500mL with distilled or deionized water.
4. Other reagents are ready for use.

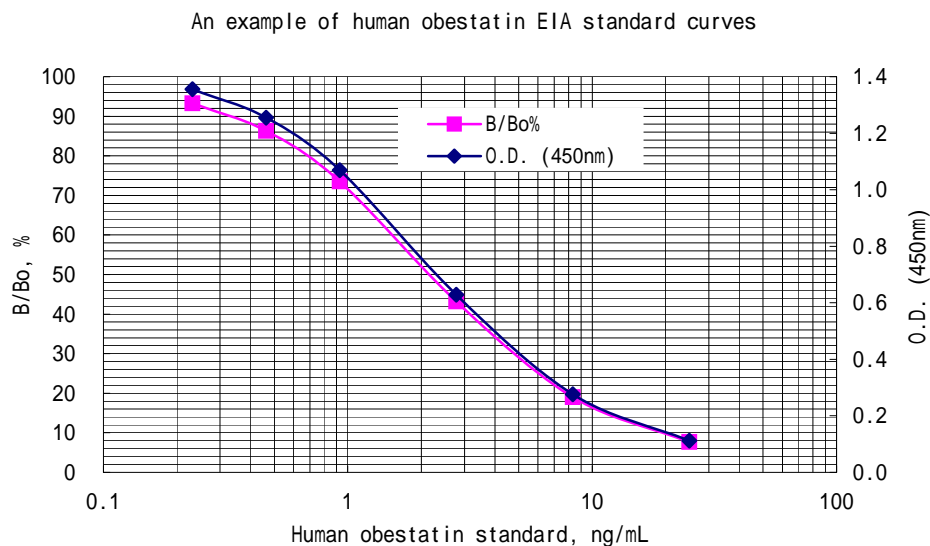
< Procedure >

1. Add 300 $\mu$ L 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.**
2. Fill 50 $\mu$ L of labeled antigen solution into each well first, then introduce 20 $\mu$ L of each of standard solutions (0, 0.231, 0.463, 0.926, 2.778, 8.333, 25ng/mL) or samples and finally add 50 $\mu$ L of human obestatin antibody solution into each well.
3. Cover the plate with Adhesive Foil and incubate it at 4°C for 20 - 22 hours (still).
4. After incubation, take off the Adhesive Foil, aspirate the contents, then add 300 $\mu$ L of washing solution to each well and aspirate. Repeat the wash step for total of five times with approximately 300 $\mu$ L/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.**
5. Pipette 100 $\mu$ L of SA-HRP Solution into each well.
6. Cover the plate with Adhesive Foil and incubate it at room temperature for 1 hour with shaking.
7. Take off the Adhesive Foil, aspirate and wash the wells five times as **Procedure 4.**
8. Add 100 $\mu$ L of TMB Substrate into each well; cover the plate with Adhesive Foil and keep it for 30 minutes with shaking at room temperature under a light proof condition (please refer to **V. Notes 7.** for more information).
9. Add 100 $\mu$ L of Reaction Stopping Solution into each well to stop coloring reaction.
10. Read the optical absorbance of the wells at 450nm.
11. 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 protease inhibitors (e.g. pepstatin A 10 $\mu$ M or inhibitors cocktail) should be added to serum or plasma samples immediately after separation and kept in an ice-bath until assay. A slight arising of determination value may be observed after addition of pepstatin A. This value arising phenomena are not inelible especially when use of cocktail inhibitor in its effective concentrations. Aprotinin was observed not to change determination value, but not effectively to inhibit protease up to addition of 0.8 TIU/ml in serum tested in our recent experiment. If the sample is tested later, they should be divided aliquoted and frozen below - 30°C (for long term storage, stored in a - 80°C deep freezer). During thawing of sample before assay, it should be kept in an ice-bath and used as soon as possible. Repeated freezing and thawing of samples should be avoided.
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(%): 3.5 ~ 9.9

Inter-assay CV(%): 5.6 ~ 9.0

### < Assay range and Sensitivity >

Range: 0.231 – 25 ng/mL;

Sensitivity =  $(2 \times SD_{0\text{ng/mL}} \times 0.231\text{ng/mL}) / (O.D._{0\text{ng/mL}} - O.D._{0.231\text{ng/mL}})$

### < 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 folds and 4 folds respectively.



## 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 date of manufacture.  
The expiry date is stated on the label of package.
- < **Package** >      For 96 tests per one kit including standards.

## VIII. References

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### < **Manufacturer** >

Yanaihara Institute Inc.  
2480-1 Awakura, Fujinomiya-shi  
Shizuoka, Japan 418-0011

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COSMO BIO CO., LTD.  
Inspiration for Life Science

TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN  
http://www.cosmobio.com      e-mail : export@cosmobio.co.jp  
Phone : +81-3-5632-9617      FAX : +81-3-5632-9618