

Obestatin (human/mouse/rat) EIA Kit

(Catalog # BN00712, 100 assays, Store at -20°C)

I. Introduction:

Assay Genie's Obestatin Enzyme Immunoassay (EIA) Kit is an in vitro quantitative assay for detecting Obestatin peptide based on the principle of Competitive Enzyme Immunoassay. The microplate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-Obestatin antibody, both biotinylated Obestatin peptide and peptide standard or targeted peptide in samples interact competitively with the Obestatin antibody. Uncompeted (bound) biotinylated Obestatin peptide then interacts with Streptavidin-horseradish peroxidase (SAHRP), which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-HRP complex and inversely proportional to the amount of Obestatin peptide in the standard or samples. This is due to the competitive binding to Obestatin antibody between biotinylated Obestatin peptide and peptides in standard or samples. A standard curve of known concentration of Obestatin peptide can be established and the concentration of Obestatin peptide in the samples can be calculated accordingly. The minimum detectable concentration of Obestatin is 0.1 pg/ml. The detection range for the kit is 0.1-1,000 pg/ml. The intra-Assay reproducibility is CV<10% & inter-Assay is CV<15%. This EIA kit shows no cross-reactivity with the following cytokines tested: e.g., Ghrelin, Nesfatin, Angiotensin II, NPY and APC.

II. Application:

Quantitative protein detection, establishing normal range, validation of antibody array results.

III. Specificity:

The capture antibody provided in this kit recognizes human, mouse & rat Obestatin.

IV. Sample Type:

- Serum & plasma
- Cell culture media etc.

V. Kit Contents:

Components	BN00712	Part No.
Obestatin Microplate (Item A) coated with secondary antibody, 96 wells	12 stripsx8 wells	BN00712-1
Wash Buffer Concentrate (20x) (Item B)	25 ml	BN00712-2
Standard Obestatin peptide (Item C): 10 µl/vial.	2 vials	BN00712-3
Anti-Obestatin polyclonal antibody (Item N): 5 µl/vial	2 vials	BN00712-4
Assay Diluent A (Item D) for Standard/Sample (serum/plasma) diluent (0.09% sodium azide as preservative)	30 ml	BN00712-5
Assay Diluent B (Item E) for Standard/Sample (cell culture medium or other sample types) diluent. (5x)	15 ml	BN00712-6
Biotinylated Obestatin peptide (Item F): 20 µl/vial.	2 vial	BN00712-7
HRP-Streptavidin Concentrate (Item G), 1000x concentrated	600 µl	BN00712-8
Positive control (Item M):100 µl.	1 vial	BN00712-9
TMB One-Step Substrate Reagent (Item H) 3,3',5,5'-tetramethylbenzidine (TMB) in buffer solution	12 ml	BN00712-10
Stop Solution (Item I), 0.2 M sulfuric acid	8 ml	BN00712-11

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Distilled or deionized water.

VII. Storage and Handling:

Standard, Biotinylated Obestatin peptide, and Positive Control should be stored at -20°C or -80°C (recommended at -80°C) after arrival. Avoid multiple freeze thaws. The remaining kit components may be stored at -20°C. Opened Microplate Wells and antibody (Item N) may be stored for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge. **Note:** the kit can be used within six months if the whole kit is stored at -20°C.

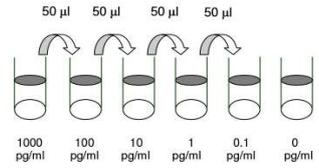
VIII. Reagent Preparation:

If testing plasma or serum samples, use Assay Diluent A to dilute Item F and Item C. If testing cell culture media or other sample types, use Assay Diluent B to dilute Item F and Item C. For sample and positive control dilutions, refer to steps 6, 7, 8 and 10 of Reagent Preparation.

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.
2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water
3. Briefly centrifuge the anti-Obestatin Antibody vial (Item N) and reconstitute with 50 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently.
4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is your anti-Obestatin antibody working solution, which will be used in step 2 of the Assay Procedure.

NOTE: the following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure).

5. Briefly centrifuge the vial of biotinylated Obestatin (Item F) before use. Add 5 µl of Item F to 5 ml of the appropriate assay diluent. Pipette up and down to mix gently. The final concentration of biotinylated Obestatin will be 10 pg/ml. This solution will only be used as the diluent in step 6 of Reagent Preparation.
6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1000 pg/ml, 100 pg/ml, 10 pg/ml, 1 pg/ml, 0.1 pg/ml and 0 pg/ml. Pipette 450 µl of biotinylated Obestatin solution into each tube, except for the 1000 pg/ml (leave this one empty). It is very important to make sure the concentration of biotinylated Obestatin is 10 pg/ml in all standards.

- a. Briefly centrifuge the vial of Obestatin (Item C). In the tube labeled 1000 pg/ml, pipette 8 μ l of Item C and 792 μ l of 10 pg/ml biotinylated Obestatin solution (prepared in step 5 above). This is your Obestatin stock solution (1000 pg/ml Obestatin, 10 pg/ml biotinylated Obestatin). Mix thoroughly. This solution serves as the first standard.
 - b. To make the 100 pg/ml standard, pipette 50 μ l of Obestatin stock solution the tube labeled 100 pg/ml. Mix thoroughly.
 - c. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration. Each time, use 450 μ l of biotinylated Obestatin and 50 μ l of the prior concentration until 0.1 pg/ml is reached. Mix each tube thoroughly before the next transfer.
 - d. The final tube (0 pg/ml Obestatin, 10 pg/ml biotinylated Obestatin) serves as the zero standard (or total binding).
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7. Prepare a 10-fold dilution of Item F. To do this, add 2 μ l of Item F to 18 μ l of appropriate assay diluent. This solution will be used in steps 8 and 10.
 8. Positive Control Preparation: Briefly centrifuge the positive control vial (Item M). To the tube of Item M, add 101 μ l 1x Assay Diluent B. Also add 2 μ l of 10-fold diluted Item F (prepared in step 7) to the tube. This is a 2-fold dilution of the positive control. Mix thoroughly. The positive control is a cell culture medium sample with an expected signal between 10% and 30% of total binding (70-90% of competition) if diluted as described above. It may be diluted further if desired, but be sure the final concentration of biotinylated Obestatin is 10 pg/ml.
 9. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
 10. Sample Preparation: Use Assay Diluent A + biotinylated Obestatin to dilute serum/plasma. For cell culture medium and other sample types, use 1X Assay Diluent B + biotinylated Obestatin as the diluent. Note: It is very important to make sure the final concentration of the biotinylated Obestatin is 10 pg/ml in every sample. EXAMPLE: to make a 4-fold dilution of sample, mix together 2.5 μ l of 10-fold diluted Item F (prepared in step 7), 185 μ l of appropriate Assay Diluent, and 62.5 μ l of your sample; mix gently. The total volume is 250 μ l, enough for duplicate wells on the microplate. Do not use Item F diluent from Step 5 for sample preparation. If you plan to use undiluted samples, you must still add biotinylated Obestatin to a final concentration of 10 pg/ml. EXAMPLE: Add 2.5 μ l of 10-fold diluted Item F to 247.5 μ l of sample.
 11. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 1000-fold with 1X Assay Diluent B. Do not use Assay Diluent A for HRP-Streptavidin preparation in Step 11.

IX. Assay Protocol:

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 μ l anti-Obestatin antibody (see Reagent Preparation step 4) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1-2 cycles/sec). You may also incubate overnight at 4°C.
3. Discard the solution and wash wells 4 times with 1x Wash Buffer (200-300 μ l each). Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 μ l of each standard (see Reagent Preparation step 6), positive control (see Reagent Preparation step 8) and sample (see Reagent Preparation step 10) into appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4°C. Discard the solution and wash 4 times as directed in Step 3.
5. Add 100 μ l of prepared HRP-Streptavidin solution (see Reagent Preparation step 11) to each well. Incubate for 45 minutes with gentle shaking at room temperature. It is recommended that incubation time should not be shorter or longer than 45 minutes. Discard the solution and wash 4 times as directed in Step 3.
6. Add 100 μ l of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).
7. Add 50 μ l of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.

X. CALCULATION:

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see calculation below) on the y-axis. Draw the best-fit curve through the standard points.

$$\text{Percentage absorbance} = (B - \text{blank OD}) / (B_0 - \text{blank OD})$$

where B = OD of sample or standard and

B₀ = OD of zero standard (total binding)

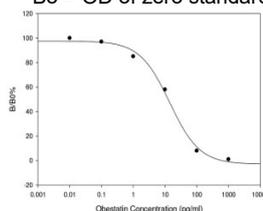


Figure: Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

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