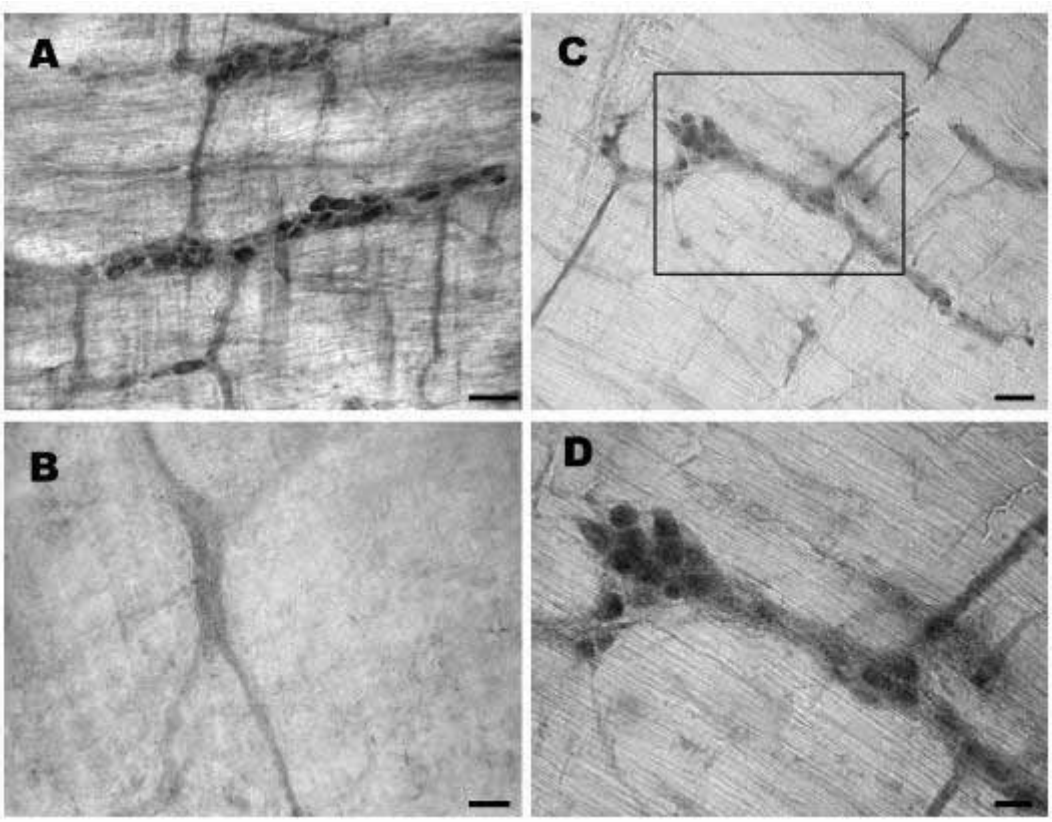
Obestatin (Rat, Mouse) - Antibody for Immunohistochemistry

Catalog#	Standard size	Price	Order
H-031-90	**100 µl**	**$450.00**	ORDER

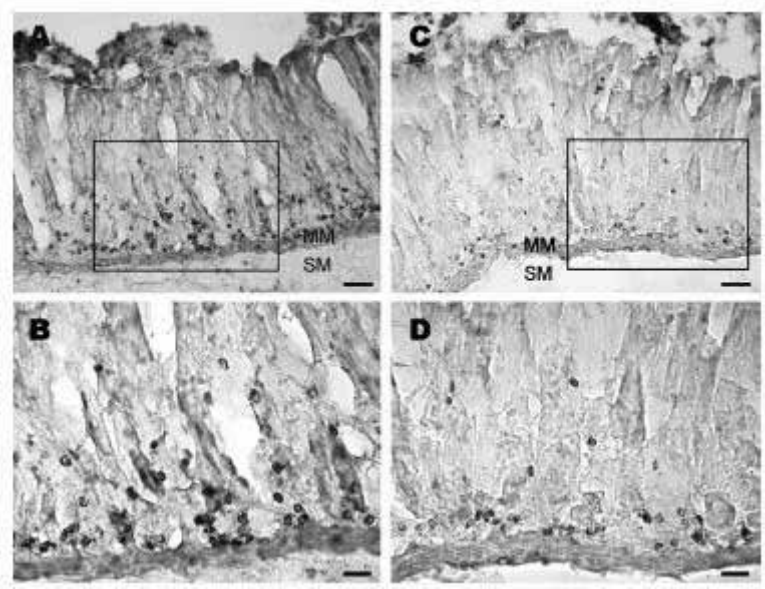
Product description | More information | References

Obestatin - Physiological Opponent of Ghrelin

Image



Obestatin- and preproghrelin-immunoreactive cells in the rat myenteric plexus. (A) Several myenteric ganglia contain clusters of obestatin-immunoreactive cells. (B) Immunoreactivity is not detected in myenteric plexus processed with obestatin antiserum pre-absorbed with the peptide (1 µg/ml) overnight. (C) Several myenteric ganglia contain preproghrelin-immunoreactive cells of varying intensities. (D) A higher magnification of the area shown in C, where several preproghrelin-immunoreactive ganglion cells are clearly seen. Scale bar: A-C, 50 mm and D, 25 µm. Dun et al. J Endocrinol. 2006 Nov;191(2):481-9.



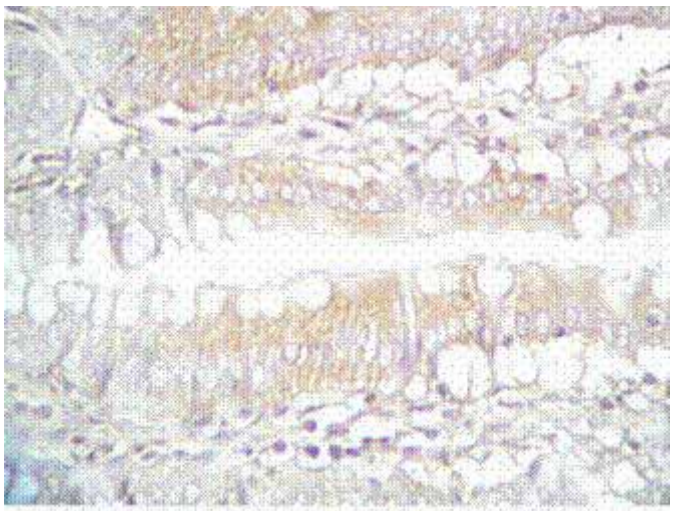
Longitudinal strips of rat stomach labeled with obestatin- or preproghrelin antiserum. (A) Obestatin-immunoreactive cells occur mostly in the glands of gastric mucosa. (B) A higher

magnification of the area in A where strongly labeled cells are seen in the glandular base. (C) Preproghrelin-immunoreactive cells are localized near the base of the glands. (D) A higher magnification showing immunoreactive cells localized mainly to the glandular base. MM, muscularis mucosae and SM, submucosa. Scale bar: A and C, 100 μm; B and D, 25 μm. Dun et al. J Endocrinol. 2006 Nov;191(2):481-9.

Immunohistochemical procedures

Animals were anesthetized with urethane (1.2 g/kg, i.p.) and intracardially perfused with 0.1 M PBS followed by freshly prepared 4% paraformaldehyde/0.2% picric acid in PBS. A segment of stomach, small and large intestine were removed, postfixed for 2 h, and stored in 30% sucrose/PBS solution overnight. In preparing the myenteric plexus, layers of smooth muscles were scraped as much as possible by cotton swabs and the plexus was processed as a whole mount (Dun et al. 1997). In single staining, tissues were processed for irOBS by the avidin-biotin complex procedure (Brailoiu et al. 2005a,b). Tissues were first treated with 3% H2O2 to quench endogenous peroxidase, washed several times and blocked with 10% normal goat serum. Tissues were then incubated in obestatin- or preproghrelin antiserum for 48 h at 4 8C with gentle agitation. Obestatin antiserum, a rabbit polyclonal directed against the rat/mouse obestatin (catalog No.: H-031-90) (FNAPFDVGIKLSGAQYQQHGRAL-NH2; Phoenix Pharmaceuticals, Inc., Belmont, CA, USA), was used at a dilution of 1:1000 with 0.4% Triton X-100 and 1% BSA in PBS. Rabbits were immunized with obestatin conjugated to Q3 thyroglobulin by the N-ethyl-N0-(3-dimethylaminopropyl) carbodiimide method. Preproghrelin antiserum, a rabbit polyclonal directed against the human preproghrelin (86-117) (Catalog No.:H-031-36) LSGVQYQQHSQALGKFLQDILWEEAKEAPADK; Phoenix Pharmaceuticals, Inc.), was used at a dilution of 1:750-1000. After thorough rinsing, sections were incubated in biotinylated anti-rabbit IgG (1:150 dilution, Vector Laboratories, Burlingame, CA, USA) for 2 h. Sections were rinsed with PBS and incubated in avidin-biotin complex solution for 1 h (1:100 dilution, Vector Laboratories). Following several washes in Tris-buffered saline, sections were incubated in 0.05% diaminobenzidine/0.001% H2O2 solution and washed for at least 2 h with Tris-buffered saline. Sections were mounted on slides with 0.25% gel alcohol, air-dried, dehydrated with absolute alcohol followed by xylene, and coverslipped with Permount. To establish the specificity of obestatin- or preproghrelin antiserum, myenteric sections were processed with obestatin- or preproghrelin antiserum pre-absorbed with the rat/ mouse obestatin peptide or preproghrelin (86-117; 1 mg/ml) overnight.

In the case of double-labeling experiments, tissue sections were first blocked with normal goat serum (1:10 in PBS, 0.5% BSA, 0.4% Triton X-100) and then incubated in obestatin antiserum (1:750 dilution) for 48 h in a cold room, with gentle agitation. Following several washes with PBS, sections were incubated with biotinylated anti-rabbit IgG (1:50, Vector Laboratories) for 2 h. After several washes with PBS, tissues were incubated with Avidin Texas Red for 3 h. After rinsing with PBS for 1 h, tissues were blocked with normal donkey serum, and incubated with choline acetyltransferase (ChAT) antiserum (1:1000; a guinea pig polyclonal from Bachem Bio Sciences, Inc., King of Prussia, PA, USA) for 48 h in a cold room. After washing with PBS for 30 min, tissues were incubated in fluorescein isothiocyanate (FITC)- conjugated Affini Pure donkey anti-guinea pig IgG (1:50, Q4 Jackson Laboratories) for 3 h. Sections were washed for 1 h with PBS, mounted in Citifluor and coverslipped. Sections were examined under a confocal scanning laser microscope (Leica TCS SL) with excitation/emission wavelengths set to 488 nm/520 nm for FITC and 543 nm/620 nm for Texas Red in the sequential mode.Dun et al. J Endocrinol. 2006 Nov;191(2):481-9.



Mouse intestine tissue was stained by Rabbit Anti-Obestatin (Mouse, Rat) Serum (Catalog No.: H-031-90)

Protocol for Immunohistochemistry:

Tissue Sample	mouse intestine, stomach tissue
Fixative	10% Formalin
Embedding	Paraffin
Negative control	Pre-immuno serum
Pretreatment	Intact
Blocking	2% Normal Goat Serum
Primary Antibody	Rabbit Anti-Obestatin (Mouse, Rat) Serum (Catalog No.:H-031-90)
Optimal Dilution	1:250~500 (1hour at RT)
Secondary Antibody	Goat anti-Rabbit IgG, Biotinylated (1:400, 30 min)
Amplification	ABC (Vector) (1:400, 30 min)
Detection System	HRP
Substrate	DAB (Sigma), 3 min
Counterstained	Hematoxylin, 30 Sec

Catalog # H-031-90

Standard Size 100 µl

Sequence Phe - Asn - Ala - Pro - Phe - Asp - Val - Gly - Ile - Lys - Leu - Ser - Gly - Ala - Gln - Tyr - Gln - Gln - His - Gly - Arg - Ala - Leu - NH2

Species Rat, Mouse

Host Rabbit

Reconstitution For consistent and reproducible results, reconstitute with 100µl of distilled water for the equivalent of undiluted antiserum, immediately before use. For the antiserum, add additional buffer.

Storage Condition For optimal results, use the antibody as soon as possible after reconstitution. Store in lyophilized form unless needed and reconstitute immediately before use. Once reconstituted, the antibody should be stable for a few days at -4°C. For storage up to a few months, prepare small aliquots after reconstitution and freeze at -20°C or -80°C. Repeated freeze thaw cycles should be strictly avoided.

Content This vial contains 100µl of Rabbit Anti- Obestatin (Rat, Mouse) Serum in the lyophilized form.

Recommended Dilution Factor
Immunohistochemistry: 1: 500-1000
Indirect ELISA: 1: 6400

Cross Reactivity

Peptide	*% Cross-reactivity*
Obestatin (Mouse, Rat)	100
Des (1-10), Obestatin (Mouse, Rat)	100
Obestatin-Gly, (Mouse, Rat)	0
Obestatin (Human, Monkey)	0
Obestatin (Porcine)	0
Obestatin (Canine)	0
Ghrelin (Mouse, Rat)	0
Ghrelin (Human)	0
Ghrelin, prepro (101-117) (Human)	0
Ghrelin, prepro (52-75) (Human)	0