

Product Information

ANTI-OREXIN A

Developed in Rabbit, IgG Fraction of Antiserum

Product Number **O9756**

Product Description

Anti-Orexin A is developed in rabbit using a synthetic peptide corresponding to the N-terminus of human orexin A (amino acids 1-17 with disulfide bridge between cysteines 6-12 and 7-14 and C-terminally added Gly-Lys), conjugated to KLH as immunogen. This sequence is identical in mouse and rat orexin A and is different from the orexin B sequence. Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins.

Anti-Orexin A recognizes human, rat and mouse orexin A. Applications include the detection of orexin A by ELISA and radioimmunoassay (RIA). The antibody does not cross-react with orexin B (human, mouse, rat), neuropeptide Y, α -MSH or leptin (human) by RIA.

The hypothalamus acts as a major regulatory center involved in the control of feeding behavior and energy homeostasis.¹⁻² Several neuropeptides and proteins have been shown to be involved in the regulation of these processes. Orexin A (hypocretin 1) a 33 amino acid peptide and orexin B (hypocretin 2), a 28 amino acid peptide, are both derived from a common 130 amino acid precursor, prepro-orexin, which is encoded by a gene localized to human chromosome 17q21.³⁻⁵ Orexin A and orexin B stimulate food consumption when administered intracerebroventricularly to rats.³ Orexin gene expression in the brain is highly restricted to distinct populations of neurons located in specific hypothalamic regions, including the lateral hypothalamic area (LHA), a region implicated in feeding behavior. Prepro-orexin mRNA expression is upregulated upon fasting.³ Immunocytochemical mapping of orexin A has identified a population of medium sized neurons within the hypothalamus, median eminence and ventral thalamic nuclei of the rat brain.⁶ This distribution has been confirmed in the human brain.⁷ Orexin-containing neurons diffusely innervate the entire brain, including monosynaptic projections to various regions of the cerebral cortex, limbic system, and brain stem.⁸ Orexin A and orexin B bind to and activate two closely related G protein-coupled receptors (GPCRs), termed orexin₁ (OX₁) and orexin₂ (OX₂) receptors. OX₁ receptor is selective for orexin A, whereas OX₂ receptor is a non-selective receptor for both orexin A and orexin B.¹ OX₁

and OX₂ mRNA are found in additional brain areas outside the hypothalamus, suggesting that orexin A and B may play additional regulatory roles in the central nervous system, such as arousal, locomotor activity and neuroendocrine functions.^{6,9,10} Dysfunction of the orexin peptide system has been linked to narcolepsy. Orexin knockout mice exhibit a phenotype strikingly similar to human narcolepsy patients, as well as to canarc-1 mutant dogs, which carry a deletion in the OX₂ receptor/Hcrtr2.^{11,12}

Reagents

Anti-Orexin A is supplied as an IgG fraction of antiserum in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Procedure - Second Antibody and Polyethylene Glycol Radioimmunoassay

Reagents:

- Buffer I: 0.01 M phosphate buffer, pH 7.4, containing 0.15 M NaCl, 0.3 % BSA, 0.1 % Triton X-100, 0.001 M EDTA and 0.1 % sodium azide.
- Buffer II: 0.01 M phosphate buffer saline (PBS), pH 7.4.
- Anti-Orexin A diluted with buffer I according to recommended working dilution

- Radiolabeled Tracer: Freshly prepared solution of 100-200 pg/ml [¹²⁵I]-Orexin A (Human) in buffer I using [¹²⁵I]-Orexin A with specific activity of approx. 2200 Ci/mmmole (approx. 200,000-300,000 dpm/ml).
- Peptide Standards/Cross Reactants: Prepare solution of 1 mg/ml in distilled water. Dilute to 2.5 ng/ml with buffer I and continue with serial dilutions with buffer I (i.e. 2.5 ng/ml, 1.25, 0.6, 0.3, 0.15, 0.075, 0.038 and 0.019 ng/ml).
- EDTA Solution: 0.1 M ethylenediamine tetraacetic acid disodium salt in distilled water; pH adjusted to 8.6.
- Normal Rabbit Serum: 2 % (v/v) solution of normal rabbit serum in buffer II.
- Second Antibody: Second antibody for RIA, Goat anti- Rabbit IgG (Product Number R 0881).
- Polyethylene Glycol Solution: 6 % PEG, MW approximately 6000 in Buffer II.

Procedure

1. Pipette 0.1 ml of peptide/cross reactants standards to glass tubes. Prepare a zero control and a blank tube, each containing 0.1 ml of Buffer I.
2. Add 0.1 ml antiserum to Orexin A to all tubes except the blank tube; to this add 0.1 ml buffer I.
3. Incubate at 4 °C for 18 to 24 hours (overnight).
4. Add 0.1 ml of [¹²⁵I]-Orexin A (Human) solution (10 to 20 pg/tube) into all tubes.
5. Prepare two empty tubes for total count. Add 0.1 ml of [¹²⁵I]-Orexin A solution to these and put them aside.
6. Vortex and incubate all tubes (except total tubes) at 4 °C for 18 to 24 hours (overnight).
7. Add 0.1 ml of EDTA solution to all tubes (except total tubes).
8. Add 0.2 ml of diluted normal rabbit serum to all tubes (except total tubes).
9. Mix well.
10. Add 0.1 ml of second antibody to all tubes (except total tubes). Mix well.
11. Add 0.5 ml of polyethylene glycol solution to all tubes (except total tubes). Mix well. Incubate at room temperature for 10 minutes.

12. Centrifuge all assay tubes (except total tubes) at 3000 rpm at 4 °C for 15 minutes.

13. Carefully aspirate off the supernatants.

14. Count the precipitate in a gamma counter.

Calculation:

1. For each standard tube calculate the % zero bound (B/Bo) as follows:

$$\% \text{ B/BO} = \frac{\text{cpm in standard (or sample)} - \text{cpm in blank}}{\text{cpm in zero control} - \text{cpm in blank}} \times 100$$

2. Generate a standard curve by plotting the % B/Bo against the log dose of standards (ng/ml).

Product Profile

A minimum working dilution of 1:20,000 is determined by ELISA using orexin A (human, mouse, rat).

A minimum working dilution of 1:300,000 is determined by RIA (2nd antibody and polyethylene glycol method) using 10-20 pg of [¹²⁵I]-Orexin A, (human, mouse, rat), with specific activity of approx. 2200 Ci/mmmole.

Sensitivity: at least 10 pg/tube of Orexin A.

Affinity constant Ka: at least 1.0×10^{10} L/M.

Peptide	%Cross-reactivity at 50% binding
Orexin A (human, mouse, rat)	100
Orexin B (human, mouse, rat)	≤0.01
Neuropeptide Y	≤0.01
α-MSH	≤0.01
Leptin (rec.human)	≤0.01

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration test.

References

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