

RayBiotech, Inc.

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Certificate of Analysis and Data Sheet

Recombinant human leptin (hLEP) antagonist (triple mutant)

Source: E.Coli	Catalog No. IP-01-352
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Background:

Leptin inhibits food intake and stimulates energy expenditure. Leptin also has thermogenic actions and regulates enzymes of fatty acid oxidation. Severe hereditary obesity in rodents and humans is caused by defects in leptin production. In addition to its critical role in the physiologic regulation of body weight leptin has a variety of other physiologic and pathologic functions resembling those of cytokines. These functions include the regulation of hematopoiesis, angiogenesis, wound healing, inflammation, and immune responses.

Description :

Recombinant human leptin, one polypeptide chain containing 146 amino and additional Ala at N-terminus acids and having a molecular mass of ~ 16 kDa, hLEP was mutated, resulting in L39A/D40A/F41A mutant was purified by proprietary chromatographic techniques.

Physical Appearance:

Sterile Filtered White lyophilized (freeze-dried) powder.

Formulation:

The protein was lyophilized from a concentrated (1mg/ml) solution with 0.0045mM NaHCO₃.

Solubility:

It is recommended to reconstitute the lyophilized hLEP mutant in sterile 0.4% NaHCO₃ adjusted to pH 8-9, not less than 100µg/ml, which can then be further diluted to other aqueous solutions.

Stability:

Lyophilized hLEP mutant although stable at room temperature for several weeks, should be stored desiccated below -18°C. Upon reconstitution at > 0.1 hLEP mutant mg/ml and up to 2 mM and filter sterilization hLEP mutant can be stored at 4°C or even room temperature for several weeks making it suitable for long term infusion studies using osmotic pumps. At lower concentration addition of a carrier protein (0.1% HSA or BSA) is suggested.

Please avoid freeze-thaw cycles.

Purity:

Greater than 98.0% as determined by:

- Gel filtration analysis.
- Analysis by reducing and non-reducing SDS-PAGE Silver Stained gel.

Amino acid sequence:

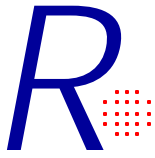
The sequence of the first five N-terminal amino acids was determined and was found to be Ala-Val-Pro-Ile-Gln

Dimers and aggregates:

The purified hLEP triple antagonist (16K) consists of > 95% monomers as determined by gel-filtration chromatography.

Biological Activity:

humanLEP triple antagonist is capable of inhibiting leptin-induced proliferation of BAF/3 cells stably transfected with



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the long form of human leptin receptor. It also inhibits various leptin effects in several in vitro bioassays.

Endotoxin:

Less than 0.1 ng/μg (IEU/μg) of human LEP triple antagonist

Protein content:

Protein quantitation was carried out by UV spectroscopy at 280 nm using the absorbency value of 0.88 as the extinction coefficient for a 0.1% (1mg/ml) solution at pH 8.0. This value is calculated by the PC GENE computer analysis program of protein sequences (IntelliGenetics).

Usage:

This material is offered for laboratory research.