ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

**Human Recombinant Purified Leptin Triple mutant Antagonist Protein**

☐ **Cat #** LEP21-TM-50 Human Recombinant (E.Coli) Purified Leptin Triple mutant Antagonist Protein **SIZE**: 50 ug

Obesity, a common nutritional disorder, is associated with diabetes, hypertension, hyperlipidemia, cancer and many other health related problems. At least five genes, Obese (ob), diabetes (db), fat (fat), agouti yellow (Ay), and tubby (tub) have been linked to obesity. Recently, Ob genes (mouse and human) have been cloned. Obese gene encodes an adipocyte-tissue derived secreted Ob protein/Leptin (167 amino acid, ~16 kDa) that controls body weight homeostasis. Exogenous administration of recombinant Ob protein can reduce food intake and body weight. However, Ob protein had no effect in db/db mice suggesting a defect in leptin signaling mechanism. Purified human Leptin (1-10 ug/gm of body weight; daily i.p injections for 14 days) has been shown to be biologically active in reducing body weight and food consumption in ob/ob and NZO mice (2).

Recently, Salomon et al (3) reported the development of a series of recombinant leptin analogs in which alanine was substituted for amino acids expressed in positions (39-41, L39A/D40A/F41A ; triple mutants) or 39-42 (L39A/D40A/F41A/I42A; quadruple mutants) of wild-type leptin. Leptin mutant showed normal binding to leptin receptor. All leptin mutants formed a 1:1 complex with chicken leptin binding domain, (chLBD) and bound chLBD or membrane-embedded leptin receptor with affinity identical to WT leptins. Muteins were devoid of any biological activity in several bioassays but were potent competitive antagonists. Howerver, these substitutions resulted in compounds with antagonist effects on leptin induced transcriptional changes observed in a heterologous cell system expressing the long form of the leptin receptor (Ob-Rb). Daily intracerebroventricular co-injection of Leptin triple mutant with exogenous leptin significantly attenuated leptin induced reduction of 48-h food-intake and body weight (4).

## Source and Properties of Leptin

Leptin is ~16 kDa, adipocyte derived, secreted protein (1). Recombinant human leptin, containing 146 amino and an additional Ala at N-terminus acids was mutated, resulting in L39A/D40A/F41A, triple mutants was cloned, expressed in E. Coli and purified (>95% by SDS-PAGE). Recombinant leptin mutant has a predicted mol. Mass of ~16 kDa. The identity of protein has been confirmed by N-Terminal sequence analyses. Endotoxin level in the final preparation is less than 10 pg/μg of Leptin.

Purified leptin protein has <5% aggregates or dimers. The sequence of the first five N-terminal amino acids was determined and was found to be Ala-Val-Pro-Ile-Gln.

**Biolgoical activity:**

HumanLEP triple antagonist is capable of inhibiting leptin-induced proliferation of BAF/3 cells stably transfected with the long form of human leptin receptor. It also inhibits various leptin effects in several in vitro bioassays.

## Form & Storage

Recombinant Leptin is supplied in either isotonic PBS solution (1 mg/ml or lot sp conc stated on the vial) or Lyophilized in 0.004 mM NaHCO3 with no preservative.

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. It may be desirable to add protease-free BSA or human serum albumin (0.1%) and solution sterile filtered. It is stable at 4oC for 2-4 weeks and 3-6 months at –70oC. Avoid repeated freeze and thaw and store in suitable size aliquots.

**General References**:

(1)Zhang, Y et al (1994) Nature 372, 425-431; (2) Pelleymounter MA et al (1995) Science 269, 540; (3) Salomon G (2006) Protein Exp. Purif. 47, 128-136; Solomon G (2006) Ann NY Acad Sci. 1091, 531-539; (4) Peters JH (2007) Endocrinol. in press.

**List of publications using ADI Leptin** (updates at the web site).

Iida M et al, 1998 Regulatory peptides 277, 77, 141-146
Tatsuya, Y, 1997 Diabetes. 46(6):1077-1080.

This product is for In vitro research use only. NOT FOR DRUG USE.

## Related material available from ADI

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| LEP11-R-1000 | Mouse Recombinant Purified Leptin Protein |
| LEP14-R1000 | Human Recombinant Purified Leptin Protein |
| LEP14-S | Anti-Human Leptin Protein antiserum # 2 |
| LEP14-M | Monoclonal Anti-Human Leptin Protein |
| LEP14-R-1000 | Rat Recombinant Purified Leptin Protein |
| LEP14-R-50 | Ovine Recombinant Purified Leptin Protein |
| LEP15-R-50 | Bovine Recombinant Purified Leptin Protein |
| LEP16-R-50 | Porcine Recombinant Purified Leptin Protein |
| LEP17-R-50 | Horse Recombinant Purified Leptin Protein |
| LEP18-R-50 | Chicken Recombinant Purified Leptin Protein |
| LEP19-R-50 | Dog Recombinant Purified Leptin Protein |
| LEP20-R-50 | Rabbit Recombinant Purified Leptin Protein |
| LEP21-TM-100 | Human Leptin Triple Antagonist Protein |
| LEP22-QM-100 | Human Leptin Quadruple Antagonist Protein |
| LEP23-TM-100 | Mouse Leptin Triple Antagonist Protein |
| LEP24-TM-100 | Rat Leptin Triple Antagonist Protein |
| LEP25-TM-100 | Ovine Leptin Triple Antagonist Protein |
| LEP26-QM-100 | Ovine Leptin Quadruple Antagonist Protein |
| LEPBP11-S | Anti-Human Leptin binding Protein antiserum |
| LEPBP15-R-100 | Human Leptin binding Protein |
| LEPBP16-R-100 | Chicken Leptin binding Protein |

Antibodies to Leptin (OBRa/b receptors), **Leptin ELISA kits**
Antibodies to Adiponectin (**Acrp30), Resistin and ELISA kits**

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