ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Human Recombinant Purified Leptin binding domain

Cat # LEPBP15-R-50 **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).

The leptin receptor (LR), encoded by the db gene is a member of the class I cytokine receptor family. It has no intrinsic kinase activity and depends on cytoplasmic-associated Janus kinase 2 (JAK2) for signalling. The extracellular part of the receptor contains several structural domains. Amino-terminally, there is a cytokine receptor homology (CRH) module, termed CRH1, which is formed by two sub-domains that have a fibronectin type III (FNIII) fold (residues 62-178 and 235-328 in the human leptin receptor). Residues 329-427 (all numbering refers to the human leptin receptor) adopt an immunoglobulin (Ig)-like fold. The next two FNIII-like sub-domains (residues 428-535 and 536-635, respectively) form a second CRH module, called CRH2. Membrane-proximally, there are two more FNIII domains. CRH2 has been identified as the main high-affinity binding site for leptin on the LR (3).

The purified leptin-binding domain (LBD) (428-635) exhibited the predicted β structure, was capable of binding human, ovine, and chicken leptins, and formed a stable 1:1 complex with all mammalian leptins and Leptin triple and quadruple mutants (4). LBD blocked leptin-induced, but not interleukin-3-induced, proliferation of BAF/3 cells stably transfected with the long form of human leptin receptor. Two main residues, Phe-500, located in loop L3, and Tyr-441, located in L1, may play a critical role in leptin binding.

Source and Properties of Leptin

Recombinant human leptin binding domaim (LBD, 208-aa) was cloned, expressed in E. Coli and purified (>95% by SDS-PAGE). Recombinant human LBD has a mol. Mass of ~24.5 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 binding domaim protein has <5% aggregates or dimers. The sequence of the first four N-terminal amino acids was determined and was found to be Ala-Thr-Pro-Val.

Biological Activity

Leptin Binding Domain recombinant human is evidenced by high affinity binding of mammalian leptins at 1:1 molar ratio.

Form & Storage

Recombinant Leptin binding domain 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) Fong Tm (1998) Mol. Pharamacol. 53, 234-240; Sandowski Y (2002) JBC 48, 46304-46309; Peelman F (2004) JBC 279, 41038-41046; Zabeau L (2004) Mol. Endocrinol. 18, 150-161; (4) Salomon G (2006) Protein Exp. Purif. 47, 128-136; Solomon G (2006) Ann NY Acad Sci. 1091, 531-539; Peters JH (2007) Endocrinol. in press.

Related material available from ADI

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-TR-100	Human Leptin Triple Antagonist Protein
LEP22-QR-100	Human Leptin Quadruple Antagonist Protein
LEP23-TR-100	Mouse Leptin Triple Antagonist Protein
LEP24-TR-100	Rat Leptin Triple Antagonist Protein
LEP25-TR-100	Ovine Leptin Triple Antagonist Protein
LEP26-QR-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 etc.

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