bioLEP ELISA L07
Measurement of Biologically Active Leptin

What is leptin?
Leptin is a 146 amino acid protein produced primarily by adipocytes and has a significant role in the regulation of energy homoeostasis. Leptin concentration is strongly correlated with body fat. In healthy humans low leptin levels are a signal of starvation/fasting and high levels represent high energy storage.

What is functional / biologically active leptin?
It was shown recently that occurring mutations in the leptin gene can result in leptin molecules unable to bind to the receptor: biologically inactive leptin\(^1\).

Mutated LEPTIN Hormone:
The Reason for Exceptional Obesity and Hunger?

\(\checkmark\) L07 is able to detect impaired leptin bioactivity in patients with a homozygous gene defect and in heterozygous carriers of such mutations\(^2\)

\(\checkmark\) Use L07 for preliminary investigation to confine the active treatment group before further investigations e.g. sequencing the DNA, and for control during treatment.

\(\checkmark\) Detect deficient and biologically inactive leptin.

\(\checkmark\) Find out additional information on the biological reactivity of the circulating leptin molecules.

\(\checkmark\) Evaluate mutation rates of the leptin gene, inexpensive and fast method.

\(\checkmark\) Accelerate obesity research by improved patient stratification in clinical studies.

\(\checkmark\) Detect in combination with the Mediagnost Leptin ELISAs E07/E077 the relative amount of receptor-binding leptin easily.

Traditional leptin immunoassays won’t be able to differentiate between both forms and measure high leptin concentrations. Thus, leptin resistance due to mutated/non-functional leptin cannot be differentiated.

Only bioLEP ELISA L07 allows the detection of functional leptin: fast, easy to use and reliable.

![Graph showing leptin concentration comparison between normal and mutants](image)

How does bioLEP work?
Eucaryotic recombinant leptin-binding protein is immobilized on a microtiterplate. Thus biologically active functional leptin in the sample will bind to the receptor. The bound leptin is subsequently detected by a highly specific conjugated antibody. In case of non-functional mutated leptin in the sample, no signal will arise.


bioLEP ELISA L07, reliable results on your hands in only 4 h:

<table>
<thead>
<tr>
<th>Preparation of reagents</th>
<th>Reconstitution</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-H Standards</td>
<td>in 1 mL Dilution Buffer VP</td>
<td>-</td>
</tr>
<tr>
<td>KS1 Control Serum 1</td>
<td>in 500 µL Dilution Buffer VP, 1:21 with Dilution Buffer VP</td>
<td>-</td>
</tr>
<tr>
<td>KS2 Control Serum 2</td>
<td>in 500 µL Dilution Buffer VP, 1:21 with Dilution Buffer VP</td>
<td>-</td>
</tr>
<tr>
<td>WP Washing Buffer</td>
<td>1:20 with Dilution Buffer VP</td>
<td>-</td>
</tr>
<tr>
<td>Sample dilution:</td>
<td>with Dilution Buffer VP e.g. 1:21 (at least use a 1:10 dilution)</td>
<td>-</td>
</tr>
</tbody>
</table>

Before assay procedure bring all reagents to room temperature 20-25°C.

Assay Procedure in Double Determination

1. Preparation of reagents
2. Reconstitution
3. Dilution

Assay Features of bioLEP ELISA L07:
- Recovery of International Standard WHO NIBSC 97/594 and Metreleptin: 100% in buffer and serum.
- In healthy human blood donors (n = 88) with leptin concentrations of 0.85 – 53.50 ng/mL measurement of functional leptin did not reveal any significant difference.
- Analytical sensitivity of 0.01 ng/mL.
- Kit contains recombinant standard material: 0; 0.05; 0.15; 0.5; 1.25; 2.5; 4; 6 ng/mL, lyophilized, range 1 – 120 ng/mL.
- Intra- and inter-assay variance: ≤ 5 %, ≤ 10 %, respectively.

References:

2. Measurement of immunofunctional leptin to detect and monitor patients with functional leptin deficiency


We have shown that the new immunofunctional bioLEP assay presented in this study is able to detect patients with reduced leptin bioactivity due to poor receptor binding resulting from either homozygous or heterozygous mutations in the leptin gene. This functional leptin deficiency constitutes a treatable condition — consequently, diagnosis should take place as early in life as possible. The bioLEP assay is a time- and cost-effective diagnostic tool that allow proper therapy to be initiated where applicable.


1. Biologically Inactive Leptin and Early-Onset Extreme Obesity


Mutations in the leptin gene typically lead to an absence of circulating leptin and to extreme obesity. A 2-year-old boy with early-onset extreme obesity due to a novel mutation in leptin is described: The change from aspartic acid to tyrosine at amino acid position 100 (p.D100Y) leads to high levels of immunoreactive but non-receptor-binding leptin. Treatment of the patient with recombinant human leptin (Metreleptin) rapidly normalized eating behavior and resulted in weight loss.