



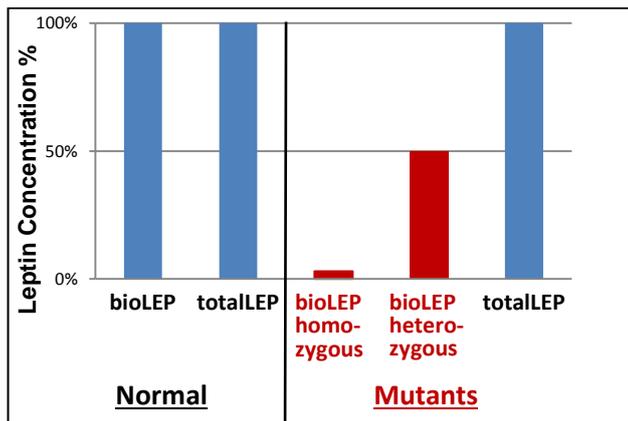
bioLEP ELISA L07 Measurement of Biologically Active Leptin

What is leptin?

Leptin is a 146 amino acid protein produced primarily by adipocytes and has a significant role in the regulation of energy homeostasis. 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 leptin¹.



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.

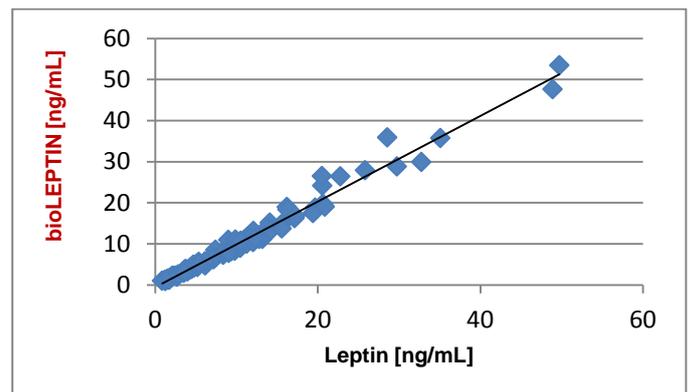
Mutated LEPTIN Hormone:

The Reason for Exceptional Obesity and Hunger?

- ✓ L07 is able to detect impaired leptin bioactivity in patients with a **homozygous gene defect** and in **heterozygous carriers of such mutations**²
- ✓ Use L07 for preliminary investigation to confine the active treatment group **before further investigations e.g. sequencing the DNA**, and **for control during treatment**.
- ✓ Detect deficient and biologically inactive leptin.
- ✓ Find out additional information on the biological reactivity of the circulating leptin molecules.
- ✓ Evaluate mutation rates of the leptin gene, inexpensive and fast method.
- ✓ Accelerate obesity research by improved patient stratification in clinical studies.
- ✓ Detect in combination with the Mediagnost Leptin ELISAs E07/E077 the relative amount of receptor-binding leptin easily.

bioLEP ELISA L07 and Mediagnost total Leptin ELISAs: excellent correlation in normal samples, see results in healthy blood donors:

$$y = 1.04x - 0.5; r^2 = 0.979; (n = 88)$$



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.

¹ Wabitsch M. et al. Biologically Inactive Leptin and Early-Onset Extreme Obesity. N Engl J Med 2015 372 (1):48-54 (see page 2)

² Wabitsch M. et al. Measurement of immunofunctional leptin to detect and monitor patients with functional leptin deficiency European Journal of Endocrinology 2017 176: 315-322 (s. page 2)



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:

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

Wabitsch M., Pridzun L., Ranke M., von Schnurbein J., Moss A., Brandt S., Kohlsdorf K., Moepps B., Schaab M., Funcke J.-B., Fischer Posovszky P., Flehmig B., Kratzsch J. 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.

European Journal of Endocrinology 2017; 176: 315–322.

¹Biologically Inactive Leptin and Early-Onset Extreme Obesity

Wabitsch M, Funcke JB, Lennerz B, Kuhnle-Krahl U, Lahr G, Debatin K, Vatter P, Gierschik P, Moepps B, Fischer-Posovszky P

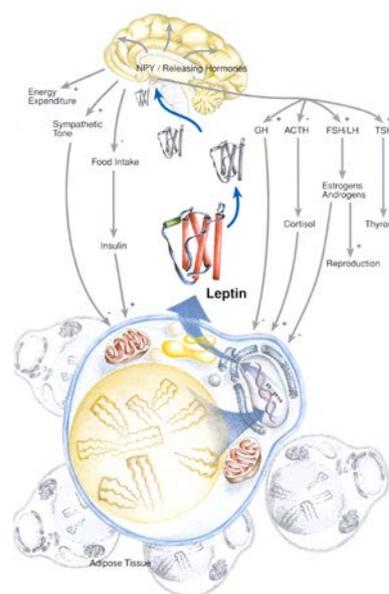
Mutations in the leptin gene typically lead to an absence of circulating leptin and to extreme obesity. A 2-year-old boy with early-onset extreme obesity due to a **novel mutation** in leptin is described: The change from aspartic acid to tyrosine at amino acid position 100 (p.D100Y) leads to high levels 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**.

New England Journal of Medicine 2015; 372(1):48-54.

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

Preparation of reagents		Reconstitution	Dilution
A-H	Standards	in 1 mL Dilution Buffer VP	-
KS1	Control Serum 1	in 500 µL Dilution Buffer VP	1:21 with Dilution Buffer VP
KS2	Control Serum 2	in 500 µL Dilution Buffer VP	1:21 with Dilution Buffer VP
WP	Washing Buffer	-	1:20 with Aqua dest.
Sample dilution: with Dilution Buffer VP e.g. 1:21 (at least use a 1:10 dilution)			
Before assay procedure bring all reagents to room temperature 20-25°C.			
Assay Procedure in Double Determination			
Pipette	Reagents	Position	
100 µL	Standard A (0.0 ng/mL)	A1/A2	
100 µL	Standard B (0.05 ng/mL)	B1/B2	
100 µL	Standard C (0.15 ng/mL)	C1/C2	
100 µL	Standard D (0.5 ng/mL)	D1/D2	
100 µL	Standard E (1.25 ng/mL)	E1/E2	
100 µL	Standard F (2.5 ng/mL)	F1/F2	
100 µL	Standard G (4 ng/mL)	G1/G2	
100 µL	Standard H (6 ng/mL)	H1/H2	
100 µL	Control Serum KS 1 (1:21 diluted)	A3/A4	
100 µL	Control Serum KS 2 (1:21 diluted)	B3/B4	
100 µL	Sample (1:21 diluted)	In the rest of the wells according the requirements	
Cover the wells with the sealing tape.			
Sample Incubation: 2 h at 20-25°C, 350 rpm			
5 x 300 µL	Aspirate the contents of the wells and wash	In each well	
100 µL	5 x with 300 µL Washing Buffer WP/ well		
100 µL	Antibody Conjugate AK	In each well	
Cover the wells with the sealing tape.			
Incubation: 1 hour at 20-25°C, 350 rpm			
5 x 300 µL	Aspirate the contents of the wells and wash	In each well	
100 µL	5 x with 300 µL Washing Buffer WP/ well		
100 µL	Enzyme Conjugate EK	In each well	
Cover the wells with the sealing tape.			
Incubation: 30 minutes at 20-25°C, 350 rpm			
5 x 300 µL	Aspirate the contents of the wells and wash	In each well	
100 µL	5 x with 300 µL Washing Buffer WP/ well		
100 µL	Substrate Solution S	In each well	
Incubation: 30 Minutes in the Dark at 20-25°C			
100 µL	Stopping Solution SL	In each well	
Measure the absorbance within 30 min at 450 nm with ≥ 590 nm as reference wavelength.			

Related Mediagnost Products for Adipokines:



- Leptin ELISA/ RIA (E07/ E077/ LEP-R44)
- Soluble LEP-Receptor ELISA (R07)
- Adiponectin ELISA (E09)
- Resistin ELISA (E50)
- Chemerin ELISA (E102)
- Progranulin ELISA (E103)
- Vaspin ELISA (E106)