Bioactive Leptin ELISA

Enzyme Immunoassay for Quantitative Determination of

Functional Leptin

For Research Use Only. Not for use in diagnostic procedures.

2-8°C

Catalog #:**L07**

Size: 96 Wells

Manufactured by

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bioLEP functional Leptin L07	96 Determinations
Regulatory Status	For Research Use Only. Not for diagnostic purposes.
Principle of the test	Ligand-Enzyme Immunoassay
Duration	< 4 h
Antibodies	specific, high-affinity polyclonal rabbit antibodies
Buffer	Ready for use
Standard	8 single standards: 0 - 6 ng/mL
Assay Range	1 ng/mL – 120 ng/mL
Samples	human Serum / Plasma (EDTA, Heparin)
Required sample volume	15 µL
Sample dilution	1:20
analytical Sensitivity	< 0.01 ng/mL
Intra- / Inter-Assay Variance	< 5% / < 9%
Linearity	1:10 – 1:120, Linear Regression $R^2 > 0.9$
Interference: Hemoglobin/ Triglyceride/ Bilirubin	No influence until to concentrations of 1 mg/mL / 100 mg/mL / 100 µg/mL
Calibration	On WHO International Standard NIBSC 97/594
Interference sOB-R	No influence until to concentration of 100 ng/mL
Reference values	Healthy adult blood donors (male / female) each 44 per gender.

1 INTENDED USE

The Eagle Biosciences Functional Leptin ELISA Assay Kit is for the quantitative measurement of functional, receptor binding Leptin levels in serum or plasma. The kit is for research use only and not for diagnostic procedures.

2 INTRODUCTION

Leptin a hormone of 146 amino acids shows a molecular weight of 16 kDa (P41159). It consists of four anti-parallel α-helices. Leptin is primarily produced by adipocytes and thus provides a signal to the energy state of the organism. By binding to the leptin receptor (P48357) it influences the activity of the JAK -STAT pathway and thereby regulates energy metabolism, especially the food intake. Leptin levels show a circadian variation (+/- 30%) and are dependent on BMI, pubertal status, and gender. In addition to the expression itself, the bioavailability of leptin, is regulated via a binding protein, as with many hormones with type I cytokine receptors. The binding protein is the extracellular domain of the Leptin receptor formed by proteolytic cleavage of the receptor be metalloproteases (ADAM 10/17).

In healthy persons food intake is reduced by increasing concentrations of leptin and by falling SOB-R quantities. In various pathological situations, this regulatory circuit is however interrupted: e.g. the leptin levels in the circulation of obese persons are increased, but resulting in no satiety. This phenomenon, known as leptin resistance, could have its cause in a reduction in the number of leptin receptors or in influencing the intracellular signal transduction by other parameters.

Lately it was shown that a naturally occurring transversion in the leptin gen (c.298G → T) results in an amino acid exchange of asparagine to tyrosin in position 100 (pD100Y). An in vitro cell culture model revealed that this mutated leptin was still secreted but unable to bind to the receptor and therewith unable to exert intracellular signaling. In vivo this mutation results in disturbance of food intake regulation and in consequence in extreme obesity. Under treatment with recombinant human leptin food intake behavior normalized and concomitantly body weight was reduced significantly.

Since the mutant leptin is recognized by the classical immunological leptin test systems, either a genetic test (sequencing of the ob gene), or a functional assay (binding to the receptor) is necessary.

The Eagle Biosciences Functional Leptin assay allows the measurement of leptin in human serum by binding it to the soluble receptor (leptin binding protein). Based on the measurement of receptor binding leptin, the bioLEP assay provides additional information on the biological reactivity of the circulating leptin molecules.

Mutations resulting in less or no binding leptin can be detected by a significant reduction of the measured bioactive Leptin. Especially in connection with measurement of total Leptin by classical immunoassays, the receptor-binding characteristics of leptin can easily be detected and quantified. This might help to evaluate mutation rates of the leptin gene by an inexpensive, fast method and accelerate obesity research by improved patient stratification in clinical studies.

3 ASSAY PRINCIPLE

Recombinant produced leptin receptor (SOB-R) is immobilized on a microtiter plate. The sample to be tested is diluted in dilution buffer and incubated on the microtiter plate; the leptin bound by the immobilized receptor is detected by a specific, polyclonal, Biotin-conjugated antibody and a streptavidin-conjugate. The quantification is performed with recombinant leptin, which is used as a standard and is traceable to the WHO International Standard (97/594).

4 WARNINGS AND PRECAUTIONS

For research and professional use only.

The Eagle Biosciences kit is suitable only for in vitro use and not for internal use in humans and animals. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. Eagle Biosciences will not be held responsible for any loss or damage (except as required by statute) howsoever caused, arising out of noncompliance with the instructions provided. Safety Data Sheet available on request

Do not use obviously damaged or microbial contaminated or spilled material.

Caution: This kit contains material of human and/or animal origin. Therefore all components and patient´s specimens should be treated as potentially infectious.

Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.

4.1 Human Serum

Following components contain human serum: **Control Sera KS1, KS2**
Source human serum for the control sera provided in this kit was tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV). No known methods can offer total security of the absence of infectious agents; therefore all components and patient´s specimens should be treated as potentially infectious.

4.2 Reagents

A-H, AK, EK, VP, WP contain as preservatives 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-4-isothiazolin-3-one (<0.015%)

H317	May cause an allergic skin reaction.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/ attention.
P302+P352	IF ON SKIN: Wash with plenty of soap and water.
P501	Dispose of contents/ container in accordance with local/ regional/ national/ international regulations.

Substrate Solution (S)
The TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbencidine (<0.05%)

H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapours/ spray.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.

Stopping Solution (SL)
The Stopping solution contains 0.2 M acid sulphur acid (H_2SO_4)

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301+P330+	IF SWALLOWED: rinse mouth.
P331	Do NOT induce vomiting.
P305+P351+	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P309+P310	IF exposed or if you feel unwell: Immediately call a POISON CENTER or doctor/physician.

4.3 General first aid procedures:

Skin contact: Wash affected area rinse immediately with plenty of water at least 15 minutes. Remove contaminated cloths and shoes.
Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.
Ingestion: After swallowing the product, if the affected person is conscious, rinse out the mouth with plenty of water: seek medical advice immediately.

5 SAMPLES

5.1 Sample Type

Serum and Plasma

Serum and Heparin/EDTA plasma yield comparable values.

5.2 Specimen collections

Use standard venipuncture for the blood sampling. Haemolytic reactions have to be avoided.

5.3 Required sample volume

15 µL

5.4 Sample stability

In firmly closable sample vials

- Storage at Room Temperature, 20-25°C: max. 2 days
- Storage at 4°C: max. 3 days
- Storage at -20°C: 2 years
- Freezer /-thaw cycles: max. 3

Freeze-thaw cycles should be minimized. Up to 3 cycles showed no effect on the measured Leptin concentration.

5.5 Interference

Neither triglycerides, bilirubin nor hemoglobin exert any influence on the measurement of Leptin in human serum up to concentrations of **100 mg/mL**, **100 µg/mL** and **1 mg/mL** respectively.

5.6 Sample dilution

- **Dilution: 1:20** with Dilution Buffer **VP**
- **Example:** Add **15 µL** Sample to **285 µL** Dilution Buffer **VP** (20 dilution factor).
- After dilution (1:20) samples may be stored at -20 ° C.
- **Minimum required sample dilution 1:10**

6 MATERIALS

6.1 Materials Provided

The reagents listed below are sufficient for 96 wells including the standard curve.

MTP	**Microtiter plate,** ready for use, coated with human Leptin receptor (rec.). Wells are separately breakable.	**(8x12) wells**
A-H	**Standards**, lyophilized, (recombinant Leptin), concentrations are given on vial labels and on the QC-certificate.	**8 x 1 mL**
KS1	**Control Serum 1,** lyophilised, (human serum), concentration is given on the QC-certificate.	**1 x 500 µL**
KS2	**Control Serum 2,** lyophilised, (human serum), concentration is given on the QC-certificate.	**1 x 500 µL**
AK	**Antibody Conjugate,** ready for use, contains biotinylated rabbit anti-hLeptin antibody**.**	**1 x 12 mL**
EK	**Enzyme Conjugate,** ready for use, contains Streptavidin-Peroxidase Conjugate**.**	**1 x 12 mL**
VP	**Dilution Buffer,** ready for use, **Please shake before use!**	**1 x 60 mL**
WP	**Washing Buffer,** 20-fold concentrated solution	**1 x 50 mL**
S	**Substrate,** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised H_2O_2 Tetramethylbencidine.	**1 x 12 mL**
SL	**Stopping Solution,** ready for use, 0.2 M sulphuric acid.	**1 x 12 mL**
-	**Sealing Tape,** for covering the **microtiter plate.**	**3 x**
↓	**Instructions for use**	**1 x**
--	**Quality Control Certificate**	**1 x**

6.2 Materials required, but not provided

- Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer **WP** (A. dest.), 950 mL.
- Precision pipettes and multichannel pipettes with disposable plastic tips
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Vortex-mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)
- Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm

7 TECHNICAL NOTES

Storage Conditions
Store the kit at 2-8°C after receipt until its expiry date. Avoid repeated thawing and freezing.

Storage Life
The shelf life of the components after initial opening is warranted for 4 weeks, store the unused strips and microtiter wells airtight together with the desiccant at 2-8°C in the clip-lock bag, use in the frame provided. The reconstituted components standards **A-H** and Control Sera **KS1** and **KS2** must be stored at –20°C (max. 4 weeks). For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Up to 3 of the freeze-thaw cycles did not influence the assay. The 1:20 diluted Washing Buffer **WP** is 4 weeks stable at 2-8°C

Preparation of reagents
Bring all reagents to room temperature (20 - 25°C) before use. Possible precipitations in the buffers have to be resolved before usage by mixing and / or warming. Reagents with different lot numbers cannot be mixed.

Reconstitution
The Standards **A – H** and Controls **KS1** and **KS2** are reconstituted with the Dilution Buffer **VP**. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer.

Dilution
After reconstitution dilute the Control Sera **KS1** and **KS2** with the Dilution Buffer **VP** in the same ratio (1:20) as the sample.
The required volume of Washing Buffer **WP** is prepared by 1:20 dilution of the provided 20fold concentrate with Aqua dest.

Assay Procedure
When performing the assay, Standards **A-H**, Control Serum **KS1 and KS2** and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, Antibody Conjugate **AK,** Enzyme conjugate **EK** as well as the succeeding Substrate Solution **S** should be added to the plate in the same order and in the same time interval as the samples. Stopping Solution **SL** should be added to the plate in the same order as Substrate Solution **S**.
All determinations (Standards **A-H**, Control Sera **KS1 and KS2** and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

Incubation
Incubation at room temperature means: Incubation at 20 - 25°C. The Substrate Solution **S**, stabilised H2O2-Tetramethylbencidine, is photosensitive–store and incubation in the dark.

Shaking
The incubation steps should be performed at mean rotation frequency of a particularly suitable microtiter plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must be adjusted. Insufficient shaking may lead to inadequate mixing of the solutions and thereby to low optical densities, high variations and/ or false values, excessive shaking may result in high optical densities and/ or false values.

Washing
Proper washing is of basic **importance** for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.
All washing must be performed with the provided Washing Buffer **WP** diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least.

The danger of handling with potentially infectious material must be taken into account.

When using an **automatic microtiter plate washer**, the respective instructions fur use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

Manual washing is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamical swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

8 SUMMARY OF THE ASSAY PROCEDURE: Functional Leptin ELISA (L07)

Preparation of reagents		Reconstitution:	Dilution
A-H	**Standards**	in **1 mL** Dilution Buffer **VP**	-
KS1	**Control Serum 1**	in **500 µL** Dilution Buffer **VP**	**1:20** with Dilution Buffer **VP**
KS2	**Control Serum 2**	in **500 µL** Dilution Buffer **VP**	**1:20** with Dilution Buffer **VP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**

Sample dilution: with Dilution Buffer **VP 1:20. Don´t use samples undiluted!**

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** (1:20 diluted)	A3/A4
100 µL	Control Serum **KS 2** (1:20 diluted)	B3/B4
100 µL	Sample (1:20 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 **5 x** with **300 µL** each Washing Buffer **WP/ well**	In each 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 **5 x** with **300 µL** each Washing Buffer **WP/ well**	In each 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 **5 x** with **300 µL** each Washing Buffer **WP/ well**	In each 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.	

9 QUALITY CONTROL

GLP requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. All kit controls must be found within the acceptable ranges as stated on the QC Certificate. If the criteria are not met, the run is not valid and should be repeated.

9.1 Quality Criteria

For the evaluation of the assay it is required that the absorbance values of the Standard A should be below 0.25, and the absorbance of standard **H** should be above **1.00.**
Samples, which yield higher absorbance values than Standard **H**, should be re-tested with a higher dilution.

10 EVALUATION OF RESULTS

10.1 Establishing of the Standard Curve

The standards provided contain the following concentrations of Leptin :

Standard	A	B	C	D	E	F	G	H
ng/mL	0.0	0.05	0.15	0.5	1.25	2.5	4	6

1) Calculate the **mean absorbance** value for the Standard A from the duplicated determination (well A1/A2).
2) Subtract the mean absorbance of the Standard A from the mean absorbances of all other samples and standards.
3) Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
4) Recommendation: Calculation of the standard curve should be done by using a computer program, because the curve is in general (without respective transformation) not ideally described by linear regression. **A higher-grade polynomial, or four parametric logistic (4-PL) curve fit** or **non-linear regression** are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).
5) The Leptin concentration in ng/mL (or pg/mL, according the chosen unit for the standards) of the samples and controls can be calculated by **multiplication** with the respective **dilution factor.**

10.2 Example of Typical Standard Curve

The exemplary data and the standard curve in Figure 2 cannot be used for the calculation of the test results. You have to establish a standard curve for each test you conduct.

Table 1 Data which describe a typical standard curve.

Standard	A	B	C	D	E	F	G	H
ng/mL	0.0	0.05	0.15	0.5	1.25	2.5	4	6
$OD_{450-620}$	0.018	0.046	0.102	0.297	0.716	1.374	2.053	2.941

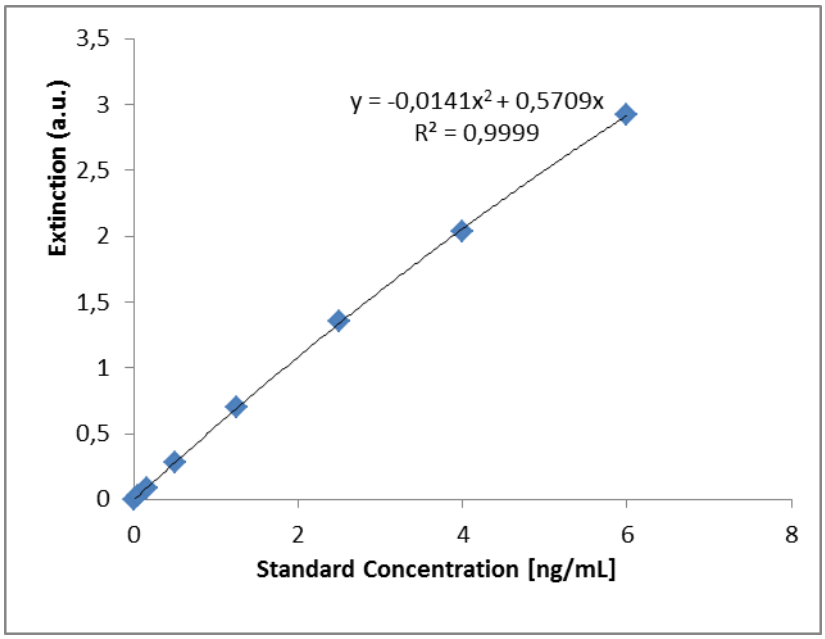


Figure 1 Exemplary Standard Curve

10.3 Evaluation of sample concentrations

Sample dilution: 1:20

Measured extinction of your sample: 0.362
Measured extinction of the Standard A: 0.018

Your measurement program will calculate the Leptin concentration of the diluted sample automatically by using the difference of sample and Standard A for the calculation. You only have to determine the most suitable curve fit. In this exemplary case the following equation is solved by the program to calculate the Leptin concentration in the sample:

$$0.344 = 0.118x^2 + 1.7122x$$
$$x = 0.61$$

If the dilution factor (**20**) is taken into account the Leptin concentration of the undiluted sample is:

$$0.61 \text{ ng/mL} \times 20 = 12.21 \text{ ng/mL}$$

11 EXPECTATION VALUES

The expected values for leptin were determined by ELISA Eagle Biosciences L07 in samples of 88 healthy subjects (44 women, 44 men) and analyzed (Table 2a). The mean quotient bioLep / Lep was 0.96 +/- 0.11SD (Range: 0.75 to 1.29).

Table 2a Expectation values of leptin for adults indicating, mean and median, the 5th and 95th percentiles.

Gender	n	AV [ng/ml]	Median [ng/ml]	SD [ng/ml]	5. Percentile [ng/ml]	95.Percentile [ng/ml]	Min. [ng/ml]	Max. [ng/ml]
female	44	15.59	12.15	11.5	1.8	39.42	1.29	53.5
male	44	5.83	4.27	5.01	1.68	17.89	0.96	19.6
Total	**88**	**10.84**	**8.44**	**10.06**	**1.68**	**39.42**	**0.96**	**53.5**

Table 2b Expectation values of quotient bioLep/totalLep. The concentration of receptor-binding and total leptin was detected in samples from 88 healthy blood donors with the Eagle Biosciences tests L07 and E07. The quotient was formed. Average value, median, the 5th and 95th percentiles, minimal and Maxilmal values are shown.

Gender	n	AV [ng/ml]	Median [ng/ml]	SD [ng/ml]	5. Percentile [ng/ml]	95.Percentile [ng/ml]	Min. [ng/ml]	Max. [ng/ml]
female	44	0.99	0,97	0.11	0.85	1.20	0.75	0.11
male	44	0.92	0,93	0.11	0.78	1.16	0.75	0.11
Total	**88**	**0.96**	**0,95**	**0.11**	**0.78**	**1.20**	**0.75**	**0.11**

12 PERFORMANCE CHARACTERISTICS

12.1 Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the Eagle Biosciences L07 is < 0.01 ng/mL. In 3 independent determinations values ranging from 0.0076 to 0.009 ng/mL were found.

12.2 Specificity

To determine the cross-reactivity with structurally similar proteins recombinant leptin, MYALEPT ™ (metreleptin) and recombinant leptin mutants D100Y and N103K, were used as samples. Recombinant leptin and Metreleptin were found to 100%, the maximum relative cross-reactivity with recombinant leptin mutants was < 0.5%.

Table 3 Specificity compared measurement Total leptin against functional leptin of leptin mutations D100Y and N103K in HEK93 transiently expressed cell culture supernatants.

	total LEP [ng/mL]	bioLep [ng/mL]	%
Leptin-Mutation D100Y	193.1	0.931	0.48
Leptin-Mutation N103K	37.1	0.031	0.09

12.3 Precision

Intra-Assay-Variance

Native human serum samples were measured repeatedly at various positions of the microtiter plate. Intra-Assay variability was on average < 5%. Exemplary results are shown in Table 4.

Table 4 Intra-Assay Variance

	Sample 1	Sample 2	Sample 3
Mean [ng/ml]	2.53	33.05	54.48
SD	0.12	0.79	1.21
CV%	4.80	2.39	2.22
n	16	16	16

Inter-Assay Variance

Serum samples were measured in independent tests. The coefficient of variation was 9% on average. Exemplary results are shown in Table 5.

Table 5 Inter-Assay Variance

	Sample 1	Sample 2	Sample 3
Mean [ng/mL]	22.36	2.54	35.46
SD	1.14	0.21	6.02
CV%	5.1	8.4	8.5
n	9	9	7

12.4 Linearity

The leptin concentration was determined at various dilutions of four human serum samples. The results shown in Figure 2 that in the samples tested no dilution effect can be demonstrated. The linearity is given from the dilution 1:10 to 1: 160.

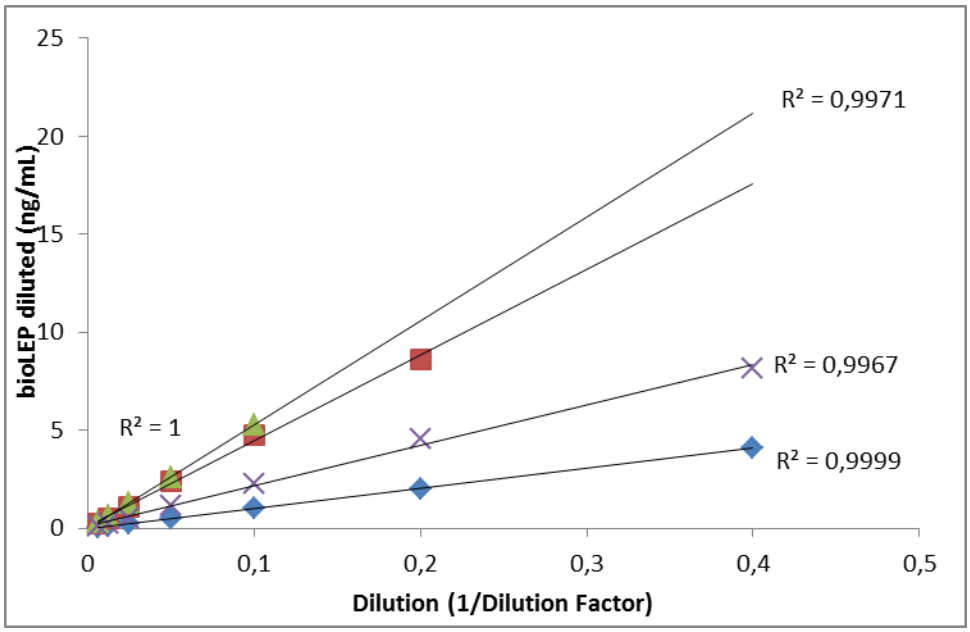


Figure 2 Linearity Leptin concentration was measured in four human serum samples diluted 1:2.5 to 1:160.

12.5 Interference

Triclycerdies, Bilirubin and Hemoglobin

The interference physiologically occurring substances was tested by adding different amounts of these potentially interfering substances to serum samples. Table 6 shows the relative recovery of leptin in comparison to the serum without additions of these substances. None of the examined substances influenced the result of the test significantly.

Table 6 Interference: None of the investigated substances affect the result significantly. The relative amount of measured leptin as compared to native serum is [%] shown.

	Triglycerides [100 mg/mL]	Bilirubin [100 µg/mL]	Hemoglobin [1 mg/mL]
Sample 1	93	108	100
Sample 2	99	107	96
Sample 3	83	99	104

Soluble Leptin Receptor (sOB-R)

Recombinant leptin was added in concentrations of 10 and 50 ng/mL to Dilutionpuffer **VP** and soluble leptin receptor (sOB-R) was added in concentrations of 20 - 100 ng/mL. The leptin contents were measured: Light sOB-R influence over mean leptin concentration was detected. This is negligible at high leptin concentration. Up to 100 ng/mL of soluble leptin receptor sOB-R does not interfere with the measurement of recombinant leptin.

Table 7 Specificity The influence of soluble leptin receptor sOB-R on the recombinant leptin (10 and 50 ng/mL) was examined. sOB-R was added in concentrations from 0 to 100 ng/mL in Dilution Buffer **VP.**

10 ng /mL Leptin			**50 ng /mL Leptin**		
sOB-R	**Recovery**		**sOB-R**	**Recovery**	
[ng/mL]	**[ng/mL]**	**[%]**	**[ng/ml]**	**[ng/mL]**	**[%]**
0	10.5	104.6	0	50.5	100.9
20	9.5	94.8	20	50.8	101.6
40	9.2	91.5	40	53.1	106.2
60	9.0	90.4	60	51.2	102.5
80	8.8	87.7	80	51.6	103.2
100	8.7	87.4	100	50.3	100.6

12.6 Recovery and traceability

Recovery of leptin international standard (NIBSC 97/594) or metreleptin was 102 and 109%, respectively. Recovery of spiked leptin or metreleptin in serum was 97 and 102 %, and leptin recovery by dilution experiments of serum was between 82 and 105 %.

Table 8 Recovery of recombinant human leptin in Buffer & Serum samples. The samples were enriched with nominal 20 ng/mL recombinant Leptin and the concentration in L07 was determined. The relative recovery in terms was calculated to the measured concentration in the buffer (17.08 ng/mL).

	Target Value	Measured Value	Recovery
	[ng/mL]	[ng/mL]	[%]
Buffer	20	17.08	85.4
Sample 1	27.71	27.36	98.7
Sample 2	23.88	24.46	102.4
Sample 3	19.14	17.72	90.2

Table 9 Recovery of Metreleptin. Meptreleptin was added to human serum serum samples and buffer in nominal concentrations of 20/25.5/12,5/6,25/3,125 ng/mL. The relative recovery was calculated in relation to leptin concentration measured in buffer.

Sample 1 Target Value [ng/mL]	Sample 1 Mesured Value [ng/mL]	Recovery [%]	Sample 2 Target Value [ng/ml]	Sample 2 Measured Value [ng/ml]	Recovery [%]
54.17	57.36	105.9	59.48	63.46	106.7
29.67	30.91	104.2	34.98	35.72	102.1
16.67	16.32	97.9	21.98	21.70	98.7
10.42	10.64	102.2	15.73	15.52	98.7
7.29	7.51	103.0	12.61	12.64	100.3

The traceability of the L07 to an International Standard WHO/NIBSC 97/594 was examined. For this purpose the standard material was diluted to 0.6; 2.0; 6.0; and 8.8 ng/ml and used as a sample in the test. The recovery in relation to the nominal content of the NIBSC standards was on averaged 96.1%.

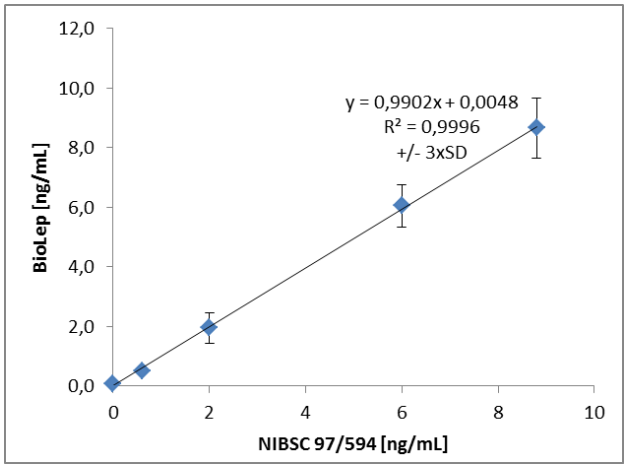
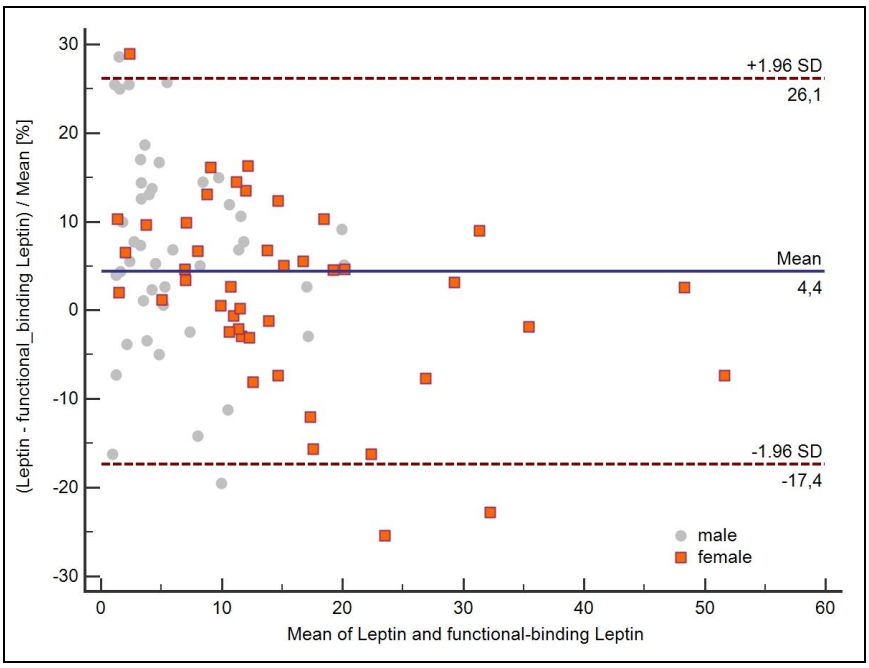


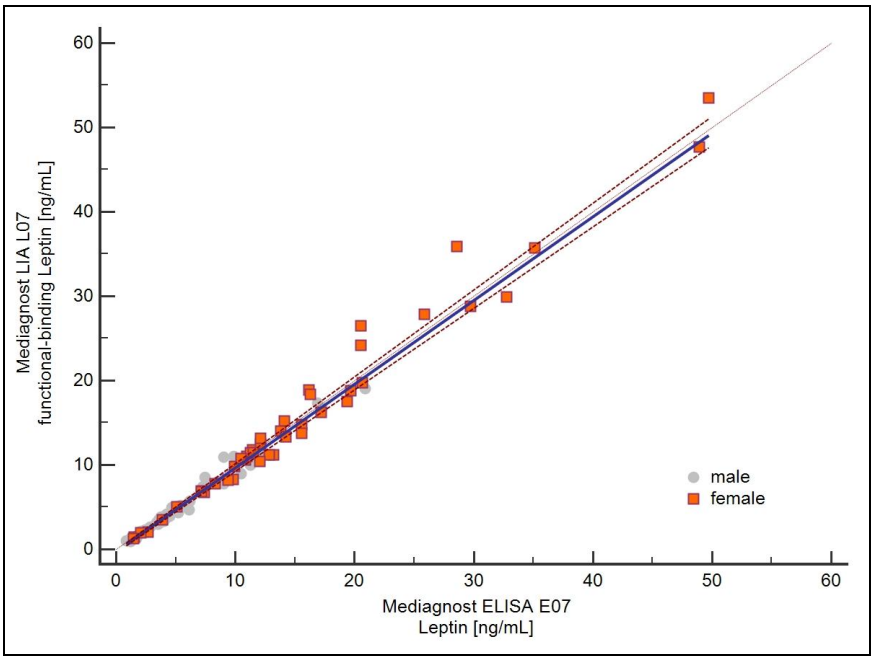
Figure 3 The traceability of bioLEP ELISA L07 on the International Standard WHO / NIBSC 97/594

13 ASSAY COMPARISON E07 L07

Results of Eagle Biosciences Functional Leptin ELISA assay (bioLEP, L07) were compared with the total Leptin ELISA (E07). Leptin concentration was measured in 88 serum samples of healthy blood donors with both test systems and results were analysed by Bland-Altman plot and Passing-Bablok regression. Results are shown in Figure 4 and prove that the mean deviation of the bioLEP assay is 4.4% of the mean of both tests. The linear relationship of both assay results is described by the Passing-Bablok curve equation of y = 0.99x – 0.21



4a) Bland-Altman-plot



4b) Passing-Bablok Regression

Figure 4 Comparison Test. Leptin [ng/mL] (n = 88) of blood donors were compared with Mediagnost E07. The results were analyzed by a) Bland-Altman plot and b) Passing-Bablok regression.

14 LITERATUR / REFERENCES

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3 Kratzsch J et al Circulating Soluble Leptin Receptor and Free Leptin Index. J Clin Endo Metab. 2002. 87(10)

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Assay Description

A-H	STD	Rec in 1 mL BUF VP	-
KS1	Control	Rec in 500 µL BUF VP	1:20 DILU BUF VP
KS2	Control	Rec in 500 µL BUF VP	1:20 DILU BUF VP
WP	WASHBUF 20x	-	1:20 DILU A. dest.
-	SPE		1:20 DILU BUF VP
-	°C 20-25 °C		

100 µL	STD A (0.0 ng/mL)	A1/A2
100 µL	STD B (0.05 ng/mL)	B1/B2
100 µL	STD C (0.15 ng/mL)	C1/C2
100 µL	STD D (0.5 ng/mL)	D1/D2
100 µL	STD E (1.25 ng/mL)	E1/E2
100 µL	STD F (2.5 ng/mL)	F1/F2
100 µL	STD G (4 ng/mL)	G1/G2
100 µL	STD H (6 ng/mL)	H1/H2
100 µL	CONTROL KS1 1:20 DILU BUF VP	A3/A4
100 µL	CONTROL KS2 1:20 DILU BUF VP	B3/B4
100 µL	SPE 1:20 DILU BUF VP	
	TAPE	
	2 h °C 20-25 ↔ 350 rpm	
5x 300 µL	5x WASHBUF WP	
100 µL	Ab AK	
	TAPE	
	1 h °C 20-25 ↔ 350 rpm	
5x 300 µL	5x WASHBUF WP	
100 µL	CONJ EK	
	TAPE	
	0.5 h °C 20-25 ↔ 350 rpm	
5x 300 µL	5x WASHBUF WP	
100 µL	SUBST TMB S	
	0.5 h °C 20-25 ▷	
	H_2SO_4 SL	
	MEASURE	