Leptin Sensitive ELISA

REF E077

IVD

Instructions for Use English

mediagnost®

Gesellschaft für Forschung und Herstellung von Diagnostika GmbH

E077_IFU_IVD_EN
Release: 29.03.2023
Revision: 001/03.2023

For in vitro diagnostic use. IVD for professional use.

Please read the instructions before use.

Leptin Sensitive ELISA E077	**96 measurements**
CE	DE/CA40/00809/21/1
Principle of the assay	Enzyme-linked immunosorbent assay
Duration (incubation time)	1.75 h
Antibody	Monoclonal antibody, ready-to-use
Buffer	Ready-to-use and 20x concentrate
Calibrators	5 individual calibrators: 0.05 - 5 µg/L, recombinant human leptin
Reference material	International standard WHO/NIBSC 97/594 recombinant leptin (15,16)
Assay range	0.01 - 50 µg/L
Control	2 controls, freeze-dried
Samples	Human serum / plasma Precise measurements even in lean individuals, e.g. anorexia or cachexia patients, adolescents and small children.
Sample volume required	25 µL
Sample dilution	1:10
Analytical sensitivity	ø 0.01 µg/L
Intra-assay / inter-assay variance	ø <10%
Reference values	Blum WF, Juul A; Reference ranges of serum leptin, In: Leptin-the voice of adipose tissue, Blum WF et al. eds. Johann Ambrosius Verlag, Heidelberg, Germany, 1997

Changes to previous version:

Change	**Description**
001	Switched to electronic instructions for use, editorial changes

Mediagnost GmbH
Aspenhaustrasse 25
72770 Reutlingen
Germany
✆ +49 7121 514840
✉ contact@mediagnost.de
www.mediagnost.de

Table of contents

All serious incidents related to the Mediagnost product Leptin Sensitive ELISA E077 must be reported to the manufacturer as well as to the competent authority in the country of residence of the user and/or patient.

Symbols and abbreviations

Symbol	Description
	Expiry date
 eIFU	Please observe the electronic instructions for use
	In vitro diagnostic medical device
	Lot number
	Manufactured by
	Order number
	Storage temperature
	Contents sufficient for x tests
	Unique device identification
	Do not expose to sunlight

Abbreviation	Description
MTP	Microtiter plate
CAL A – E	Calibrators A - E
CTR1	Control 1
CTR2	Control 2
DET	Antibody-HRP-Conjugate
DIL	Dilution Buffer
WB	Wash Buffer
S	Substrate
STP	Stop solution
CF	Cover foil
CERT	Lot certificate

1 Purpose

Quantitative measurement of human leptin in human serum or plasma.

2 Introduction

The peptide hormone leptin was first identified as the product of the ob gene in 1994 (1, 2). It has a molecular weight of 16 kDa and is thought to play a key role in regulating body weight. Leptin is almost exclusively produced by differentiated adipocytes (3-5). It acts on the central nervous system, primarily on the hypothalamus, by suppressing food intake and increasing energy consumption (2, 6-9).
In addition to its metabolic effects, leptin also has a major impact on a number of endocrine axes.
When utilizing leptin levels in a clinical context, it is important to keep in mind that they exhibit moderate circadian fluctuation, with a peak around 2 a.m. (10). Leptin levels at this time of night are approximately 30-100% higher than those measured in the morning or early afternoon. The timing of blood sample collection must take these fluctuations as well as time of food intake into account. One leptin measurement is sufficient for informative results provided that standard conditions are met, e.g. normal eating patterns and blood drawn in the morning or early afternoon.
Reference ranges are required for a valid interpretation of measured leptin levels. Because levels are most strongly influenced by body fat mass, reference ranges should be adjusted for body fat mass (body mass index, BMI, or body fat percentage measured by bioelectrical impedance analysis, BIA). Leptin levels vary in an age-dependent manner (11). In addition, women also have higher leptin levels than men even after controlling for fat mass (12, 13). Reference ranges should therefore also take sex and pubertal development into account.

This immunoassay kit enables measurement of human leptin in serum or plasma for diagnostic purposes. The test kit is also suitable for measuring exceptionally low concentrations of leptin, for example in samples from patients with anorexia or cachexia and from children.
For the standard application of measurement in serum or plasma samples from individuals with normal weight (expected levels between 1 and 100 ng/mL leptin), we recommend our Leptin ELISA product number E07.

3 Principle of the assay

The Mediagnost ELISA E077 for sensitive measurement of leptin is a type of assay called a sandwich assay which uses two specific antibodies. The first antibody is affixed to the microtiter plate and binds leptin from the sample. In the next step, the second specific anti-leptin antibody binds the leptin immobilised on the plate. A subsequent enzymatic reaction changes the colour of the substrate to blue, whose intensity is proportional to the concentration of leptin in the sample. After the reaction is stopped using acid, the intensity of the yellow colour is quantified by absorbance measurement and converted to the leptin concentration using a calibration curve.

4 Materials

4.1 Test kit contents

The reagents supplied in the test kit are sufficient for 96 tests.

Abbreviation	Description	Quantity
MTP	**Microtiter plate,** ready-to-use, Coated with mouse anti-hLeptin antibody. Individual wells can be snapped off.	8 x 12 wells
CAL A – E	**Calibrators A – E,** lyophilised (recombinant hLeptin). Concentrations are shown in ng/mL on the labels and lot certificate.	5 x 1 mL
CTR1	**Control 1**, lyophilised, (human serum), Target and acceptance range are shown in ng/mL on the lot certificate.	1 x 250 µL
CTR2	**Control 2**, lyophilised, (human serum), Target and acceptance range are shown in ng/mL on the lot certificate.	1 x 250 µL
DET	**Antibody-HRP-Conjugate**, ready-to-use, Biotinylated mouse anti-hLeptin antibody + streptavidin peroxidase conjugate.	1 x 12 mL
DIL	**Dilution Buffer,** ready-to-use	1 x 60 mL
WB	**Wash Buffer**, 20x concentrate solution	1 x 50 mL
S	**Substrate**, ready-to-use, horseradish peroxidase (POD) substrate, stabilised tetramethylbenzidine.	1 x 12 mL
STP	**Stop solution,** ready-to-use, 0.2 M sulfuric acid.	1 x 12 mL
CF	**Cover foil** for the **microtiter plate**	2 x
CERT	**Lot certificate**	1 x

4.2 Required materials not supplied

- Deionised water or distilled water (Aqua destillata, A. dest), 950 mL
- Adjustable micropipettes and multichannel pipettes with disposable tips
- Vortex mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (optional)
- Photometer for microtiter plates (ELISA reader), filter for 450 nm and ≥ 590 nm
- Polypropylene (PP) / polyethylene (PE) tubes for sample dilution

5 Warnings and precautions

This Mediagnost kit is for in vitro diagnostic use only and is not suitable for internal use in humans or animals. This product is intended for use by trained professionals only and may only be used exactly as described in the instructions for use provided. Unless otherwise specified by law, Mediagnost GmbH shall not be held liable for any losses or damages arising from failure to comply with the instructions for use. A safety data sheet can be found at www.mediagnost.de or provided upon request.

CAUTION: This kit contains material of human and/or animal origin. Treat the components of the kit as potentially infectious.

Controls CTR1 and CTR2 contain material of human origin. The human material used to prepare this product tested negative to several relevant pathogens, such as human immunodeficiency virus (HIV) RNA and hepatitis C virus (HCV) RNA. Tests for hepatitis B surface antigen (HBsAg), as well as for antibodies to human immunodeficiency virus I and II (anti-HIV I and II), for hepatitis B core antigen (anti-HBc) and for hepatitis C virus (anti-HCV) were also negative. However, since no test can completely exclude the presence of a pathogen, the reagents should be treated as potentially infectious material.

Please do not use any expired, obviously damaged, microbially contaminated, or spilled reagents. In case of such reagents, please write to contact@mediagnost.de and keep the reagents for a potential return. Components may not be exchanged between lots.

Always take appropriate precautions and follow the rules of good laboratory practice when storing, using and disposing of the components of the kit. The components of the kit must be disposed of in accordance with local regulations.

5.1 Reagents

CAL, CTR, DET, DIL contain:

Hazard pictograms (CLP):

GHS07

Signal word (CLP):	Warning
Hazardous ingredients:	2-methyl-2H-isothiazol-3-one, reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1)
Hazard statements (CLP):	H317 - May cause an allergic skin reaction. H412 - Harmful to aquatic life with long lasting effects.
Precautionary statements (CLP):	P261 - Avoid breathing mist/vapours/spray. P273 - Avoid release to the environment. P280 - Wear protective gloves/protective clothing/eye protection. P333 + P313 - If skin irritation or rash occurs: Get medical advice/attention. P362 + P364 - Take off contaminated clothing and wash it before reuse. P501 - Dispose of contents/container in accordance with national regulations.

6 Samples

6.1 Sample material

Human serum or plasma is a suitable sample material.
No significant differences in leptin concentration have been observed between serum samples and EDTA plasma samples.

6.2 Blood drawing / sample collection

The blood used for sample collection should be withdrawn using standard venipuncture by qualified staff. Avoid haemolysis during sample collection.
Human serum or plasma samples should be collected in the morning or early afternoon from patients who have eaten normally.
Leptin concentrations exhibit circadian fluctuation, with a peak around 2 a.m. (37). The timing of blood sample collection should take these fluctuations as well as time of food intake into account.

6.3 Sample volume required

A single measurement requires a minimum of 10 µL sample, while duplicate measurements require at least 20 µL. Depending on the specific procedure used, a higher sample volume may be necessary to ensure correct and safe handling.

6.4 Sample stability

Samples should be stored in suitable sample vessels which can be sealed tightly.

A storage temperature of -20°C or below is recommended for long-term sample storage. Storing samples for 2 years at -20°C was found to have no impact on measured values. Freezing and thawing of samples should be minimised. 5 freeze/thaw cycles were found to have no impact on samples.

Storage temperature	Duration of storage
20 – 25 °C	max. 2 days
2 – 8 °C	max. 3 days

6.5 Interference

No effect on measurements was observed from haemoglobin, triglycerides or bilirubin in the sample at concentrations of up to **1 mg/mL, 100 mg/mL** or **200 µg/mL**, respectively. The user should test the use of haemolytic, lipaemic or icteric samples in advance.

6.6 Sample dilution

Samples must be diluted 1:10 in Dilution Buffer **DIL**. Samples can and should be diluted less, up to a minimum of 1:5, if values below 0.5 ng/mL leptin are expected or reached. Samples can and should be diluted more if values greater than 50 ng/mL are expected or reached. See Table 13 for details.

6.6.1 Dilution protocol example

For a duplicate measurement, pipette e.g. 225 µL Dilution Buffer **DIL** into PE/PP tubes and add 25 µL serum or plasma (corresponding to a 1:10 dilution). Mix this solution and use 2 x 100 µL (100 µL per well) in the assay.

7 Technical notes

7.1 Preparing the reagents

All kit components must be brought to **room temperature** (20-25°C) **before use**. Some buffers may contain precipitates, which must be re-dissolved before use by mixing and heating. Reagents from kits with different lot numbers may not be mixed.

7.2 Reconstitution

The calibrators **A - E** and controls **CTR1 and CTR2** are reconstituted using the Dilution Buffer **DIL** supplied with the kit. For reconstitution, the reagents must be incubated for 15 minutes at room temperature and then vigorously mixed using a vortex mixer. However, avoid foaming while mixing.

7.3 Dilution

After reconstitution, dilute the controls **CTR1** and **CTR2** with Dilution Buffer **DIL** at the same ratio as the samples. After reconstitution, the calibrators are ready to use and may not be diluted.

Prepare the volume of Wash Buffer **WB** required for the assay by diluting the 20x concentrate at 1:20 ratio with distilled water.

7.4 Incubation

Incubation at room temperature means: **Incubation at 20-25°C**.

The substrate **S,** stabilised tetramethylbenzidine, is sensitive to light. It must therefore be protected from light during storage and incubation.

7.5 Assay procedure

Measurements (blank, calibrators **A-E**, controls **CTR1 and CTR2**, and samples) should always be performed in duplicate. Pipette precisely and follow the test protocol exactly. Calibrators **A-E**, controls **CTR1, CTR2** and the samples should be pipetted as quickly as possible when performing the assay. The antibody-POD conjugate **DET**, substrate **S** and Stop Solution **STP** should then be added to the plate in the same order and with the same time interval. This prevents variations in concentration values due to differences in incubation times.

7.6 Shaking

The plate should be shaken at a frequency of **350 rpm** during incubation on a shaker suitable for microtiter plates. Deviations from this value are possible on a case-by-case basis depending on the design of the device; in this case, the shaking frequency must be adjusted. Too little shaking may result in lower optical densities due to insufficient mixing of the solutions, high variation and/or incorrect values, while excessive shaking may lead to higher optical densities.

7.7 Washing

Proper washing is of the utmost importance for the assay to be performed safely, correctly and precisely. Insufficient washing is a common cause of invalid assay performance. This may result in uncontrolled, nonspecific variations in the measured optical densities, which may cause incorrect calculation of the results from the samples. Potential indications of this situation include high blank values and measured values which fluctuate variably. The Wash Buffer supplied must be diluted to working concentration and used for washing. The wash volume must be at least 300 µL per well per wash cycle.

If using a special **automated microplate washer**, always follow the instructions of use for the device. The device settings must be configured to parameters including microtiter plate geometry and the parameters of the washing specification. Ensure that the dispensing and aspiration manifold on the device does not damage the surface of the microtiter plate wells. The volume of liquid remaining after each aspiration must be kept to a minimum. After the entire washing procedure is complete, check the quantity of liquid remaining and reduce it if necessary by tapping the microtiter plate several times on lint-free absorbent paper.

Manual washing is a good alternative. The wash solution can be dispensed manually by repeater pipette, multichannel pipette, or spray bottle. The wash solution can be removed by vigorously inverting the microtiter plate over a sink. If an aspiration manifold is used, ensure that the device does not damage the surface of the microtiter plate wells. After each individual wash cycle, remove all the remaining liquid by tapping the microtiter plate on lint-free absorbent paper.

8 Stability and storage

8.1 Storage conditions

Upon receipt, the test kit should be stored at 2-8°C until the expiry date.

8.2 Shelf life

The reagents have a shelf life of 4 weeks **after first opening**. Store unused **test plate** strips together with the desiccant pouch **airtight** in the resealable seal-top bag at 2-8°C. Please use only the supplied frame for the strips. Store **reconstituted components** (calibrators **A-E** and controls **CTR1** and **CTR2**) at -20°C. For further use, thaw at temperatures no higher than room temperature and do not vortex excessively. No impact on the assay was observed after up to 3 such freeze/thaw cycles. The ready-to-use Wash Buffer **WB** diluted 1:20 is stable for 4 weeks at 2-8°C.

9 Assay procedure: sequence

Preparing the reagents			
Reagent preparation		**Reconstitution**	**Dilution**
CAL A-E	**Calibrators**	each in **1 mL** Dilution Buffer **DIL**	-
CTR1 **CTR2**	**Controls**	each in **250 µL** Dilution Buffer **DIL**	**1:10** in Dilution Buffer **DIL**
WB	**Wash Buffer** 20x conc.	-	**1:20** with **dist. water**
Sample dilution: 1:10 e.g. 225 µL DIL + 25 µL sample			
Bring all **reagents** to **room temperature (20-25°C)** before performing the test.			
Perform test in duplicate			
Pipette	**Reagents**		**Position**
100 µL	Dilution Buffer **DIL** (blank)		A1/A2
100 µL	Calibrator **CAL A** (**0.05 ng/mL**)		B1/B2
100 µL	Calibrator **CAL B** (**0.5 ng/mL**)		C1/C2
100 µL	Calibrator **CAL C** (**1.5 ng/mL**)		D1/D2
100 µL	Calibrator **CAL D** (**3.5 ng/mL**)		E1/E2
100 µL	Calibrator **CAL E** (**5 ng/mL**)		F1/F2
100 µL	**CTR1** Control 1 (diluted 1:10)		G1/G2
100 µL	**CTR2** Control 2 (diluted 1:10)		H1/H2
100 µL	Sample (diluted 1:10)		pipette into remaining wells as needed
Seal the wells tightly with cover film.			
Incubation: 1 h at (20-25°C), 350 rpm			
3x 300 µL	Aspirate and wash the plate **3x** with **300 µL** Wash Buffer (diluted 1:20) per well.		In each well
100 µL	Antibody-POD conjugate **DET**		In each well
Seal the wells tightly with cover film.			
Incubation: 30 minutes at (20-25°C), 350 rpm			
3x 300 µL	Aspirate and wash the plate **3x** with **300 µL** Wash Buffer (diluted 1:20) per well.		In each well
100 µL	Substrate **S**		In each well
Incubation of substrate S: 15 minutes in the dark at (20-25°C)			
100 µL	Stop Solution **STP**		In each well
Measure absorbance at **450 nm** (reference filter ≥ 590 nm) within 30 min.			

10 Quality control

The following criteria are used to evaluate and assess the validity of an assay run:

- To ensure that the assay is evaluable, the absorbance of the blank should not be greater than 0.25 units while the absorbance of calibrator E should be at least 1.0 units.
- The measured concentrations of the controls in the kit must be within the permitted acceptance range shown on the lot certificate provided with the test kit.

If these criteria are not met, the assay is invalid and must be repeated.

The following points should also be noted:

- Samples with a measured absorbance outside the calibration range between **CAL A - CAL E** are outside the calibration curve and should be measured again at a more appropriate dilution in a second test run to obtain a reliable measurement.
- The absorbances of the calibrators which are also listed on the lot certificate are theoretical values that may not be used to calculate the concentrations of measured samples.

11 Analysis

11.1 Generation of the calibration curve

The supplied calibrators contain leptin at the following concentrations:

Calibrator	A	B	C	D	E
ng/mL	0.05	0.5	1.5	3.5	5

a) Calculate the **mean** optical density of the blank from the duplicate measurements (wells A1/A2).

b) Subtract the mean optical density of the blank from the mean optical densities of the calibrators, controls and samples.

c) Plot the calibrator concentrations (x-axis) against their measured optical densities (y-axis).

d) A statistical program should be used to calculate the calibration curve since it is typically not best described by a linear regression. **A higher-order polynomial, 4-parameter fit,** or **nonlinear regression** are suitable for this analysis; in some cases a spline or point-to-point fit may be appropriate.

e) The calibration curve is used to obtain the leptin concentration in the diluted controls **CTR1** and **CTR2** and in the diluted samples. **Multiplying** each calculated leptin concentration **by** the corresponding **dilution factor** then yields the leptin concentration of the undiluted samples / controls.

11.2 Example of a typical calibration curve

The representative data and calibration curve in Figure 1 may **not** be used to calculate assay results! A separate calibration curve must be processed for each test run.

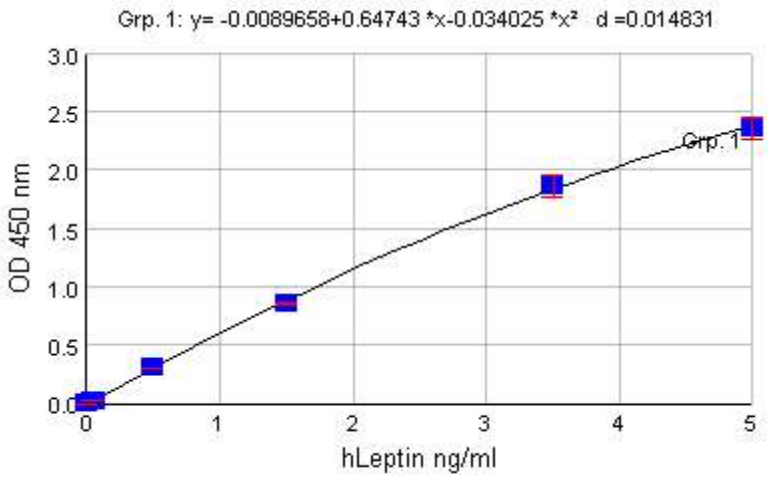

Fig. 1 Example of a calibration curve

11.3 Example calculation of leptin concentration in a sample diluted 1:10

- Measured absorbance of sample: 0.293
- Measured absorbance of blank: 0.015

Your analysis software will use the difference between the absorbance of the sample and the blank (= 0.278) and the appropriate curve fit (here: 2nd degree polynomial) to calculate the leptin concentration in the diluted sample by solving the equation in Fig. 1 for x. In this case, the leptin concentration in the diluted sample is:

$$0.278 = -0.0089658+0.64743x-0.034025x0.034025x^2$$
$$0.4524 = x$$

After correcting for the dilution factor of 1:10, the sample thus contains 4.4524 ng/mL leptin.

11.4 Interpretation of results

Therapeutic decisions should not be made solely on the basis of test results. Results should be interpreted in the context of the patient's history, other clinical observations and the results of other diagnostic tests. Moreover, we recommend that each laboratory establishes its own reference ranges.

11.5 Limitations

The Mediagnost Leptin Sensitive ELISA E077 is based on antibodies. This general method can be affected by the presence of heterophilic antibodies or rheumatoid factor in the sample. Although this impact is reduced by the assay design, it cannot be ruled out completely.

12 Reference values

Serum leptin concentration typically correlates closely with body fat mass. Leptin levels are generally low in lean individuals and high in obese individuals. There is also a clear sex difference between men (lower levels) and women (higher levels) when corrected for body fat percentage. Undergoing puberty also impacts leptin levels. These influences must be taken into account when determining expected leptin levels.
There are several different methods for determining body fat percentage, for example calculating body mass index (BMI, weight (in kg) divided by the square of height (in m)), bioelectrical impedance analysis (BIA), or total body dual energy x-ray absorptiometry (DXA). Although BMI is a less precise means of estimating actual body fat mass than more sophisticated methods such as BIA or DXA, using BMI has several advantages:

1) BMI is not influenced by the regression models used.
2) BMI is easy to determine since only height and weight are needed.
3) BMI can usually also be determined retrospectively.
4) BMI is the most precise method to measure short-term changes in fat mass, e.g. while fasting.

Based on these considerations, the following expected ranges for serum leptin concentration were developed based on BMI as the most important limiting independent variable and also incorporating sex and pubertal development (14, see Figures 2 to 9 and Tables 1 to 9 below). No significant age-dependence was observed from the age of 20 years onward. The expected values listed below by sex and age can be used to compare test results from a tested patient with leptin levels measured in healthy patients with the same BMI in order to identify pathological aberrations.

The best regressions for various population groups (see Figures 2 to 9) are obtained using the following exponential function:

$$Leptin = a \cdot e(b \cdot BMI)$$

The 5th and 95th percentiles are represented by the following equations:

$$Leptin = a \cdot e(b \cdot BMI - c)$$

and

$$Leptin = a \cdot e(b \cdot BMI + c)$$

These equations yield linear functions when plotted on a semi-logarithmic graph (y-axis = log leptin). The values for a, b and c are provided in Table 1 according to sex, pubertal development, and for adults. These values can easily be used to extend the expected leptin ranges to higher or lower BMI ranges if needed.

Example:
The 50th percentile for boys in Tanner stages 3 & 4 is represented by the following curve:

$$Leptin = 0.0181.e(0.2067 \cdot BMI)$$

The 5th percentile is: $Leptin = 0.0181\ e(0.2067 \cdot BMI - 1.1919)$
and the 95th percentile is: $Leptin = 0.0181\ e(0.2067 .BMI + 1.1919)$

In a semi-logarithmic graph, these functions represent parallel lines which are equidistant to the 50th percentile.

12.1 Calculation of the standard deviation (SD; Z-scores)

Calculation of the standard deviation is a suitable method for determining the deviation of a measured leptin concentration from the corresponding reference range. The latter is determined by comparing the measured leptin level of a patient with a given BMI to the leptin level of the corresponding sex and age group. The calculated deviation is equivalent to the x-fold standard deviation. This method allows leptin levels to be classified by BMI, sex and pubertal development/age and pooled for further analyses. The impact of parameters such as BMI, sex or age can be eliminated for further analyses in this manner.
Due to the logarithmic dependence of the leptin values, the leptin SD is calculated as follows:

$$Leptin\ SD = \frac{ln(leptin) - ln(a) - b \cdot BMI}{d}$$

In this equation, “ln” stands for the natural logarithm (to the base e). The constants a, b and d are listed in Table 1 by sex and age.

Example:
A boy in Tanner stage 3, BMI = 25 kg/m2, measured leptin concentration = 5 ng/mL:

$$Leptin\ SD = \frac{ln(5) - ln(0.0181) - 0.2067 \cdot 25}{0.6850} = 0.66$$

12.2 Estimation of the optimal sample dilution

Since serum leptin levels can vary by several orders of magnitude depending on body fat mass, an adequate sample dilution is essential for high measurement accuracy. Samples should be diluted such that the end concentrations lie within the calibration curve range. The expected ranges can be a useful tool for estimating expected leptin levels based on BMI, sex and age.

Example 1
Adult woman, BMI = 35 kg/m^2. According to the reference ranges for adult women, the average leptin level for a woman with a BMI of 35 kg/m^2 is approx. 50 ng/mL. The optimal dilution in this situation would thus be 1:20.

Example 2
Prepubescent boy, BMI = 24 kg/m^2. According to the reference ranges, the average leptin level for a boy in Tanner stages 1 and 2with a BMI of 24 kg/m^2 is approx. 10 ng/mL. The optimal dilution in this situation would thus be 1:10.

Table 1 Constants a, b, c and d for calculating the reference ranges and leptin standard deviations based on BMI. Groups of normal, healthy individuals are listed separately by sex and pubertal development stage/age. TS = Tanner stage, n = number of persons, a, b, c and d = constants

Group	n	a	b	c	d
Male					
TS 1&2	136	0.0146	0.2706	0.8821	0.5379
TS 3&4	50	0.0181	0.2067	1.1919	0.6850
TS 5	112	0.0316	0.1462	1.0821	0.6558
Adults	380	0.0130	0.2200	1.1053	0.6740
Female					
TS 1&2	136	0.0422	0.2499	0.7849	0.4786
TS 3&4	43	0.0543	0.2357	0.5745	0.3379
TS 5	157	0.2550	0.1508	0.7053	0.4301
Adults	587	0.3042	0.1467	0.8548	0.5212

Table 2 Girls in Tanner stages 1 and 2

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.22	0.30	0.66	1.45	1.99
12	0.28	0.39	0.85	1.86	2.56
13	0.36	0.50	1.09	2.38	3.29
14	0.46	0.64	1.40	3.06	4.22
15	0.60	0.82	1.79	3.93	5.42
16	0.76	1.05	2.30	5.04	6.96
17	0.98	1.35	2.95	6.47	8.93
18	1.25	1.73	3.79	8.31	11.5
19	1.61	2.22	4.87	10.7	14.7
20	2.07	2.85	6.25	13.7	18.9
21	2.65	3.66	8.03	17.6	24.3
22	3.41	4.70	10.3	22.6	31.2
23	4.37	6.03	13.2	29.0	40.0
24	5.62	7.75	17.0	37.2	51.4
25	7.21	9.95	21.8	47.8	65.9
26	9.26	12.8	28.0	61.4	84.7
27	11.9	16.4	35.9	78.8	109.0
28	15.3	21.1	46.1	101.0	140.0
29	19.6	27.0	59.2	130.0	
30	15.2	34.7	76.1		
31	32.3	44.6	97.7		
32	41.5	57.2	125.0		
33	53.2	73.4			
34	68.4	94.3			
35	87.8	121.0			
36	113.0				
37	145.0				

Table 3 Boys in Tanner stages 1 and 2

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.08	0.12	0.29	0.69	0.99
12	0.01	0.16	0.38	0.91	1.30
13	0.14	0.20	0.49	1.19	1.71
14	0.19	0.26	0.65	1.56	2.24
15	0.24	0.35	0.85	2.04	2.93
16	0.32	0.46	1.11	2.68	3.84
17	0.41	0.60	1.45	3.51	5.04
18	0.55	0.79	1.90	4.60	6.60
19	0.72	1.03	2.50	6.03	8.66
20	0.94	1.35	3.27	7.90	11.3
21	1.24	1.77	4.29	10.4	14.9
22	1.62	2.33	5.62	13.6	19.5
23	2.12	3.05	7.37	17.8	25.5
24	2.78	3.99	9.66	23.3	33.5
25	3.65	5.24	12.7	30.6	43.9
26	7.78	6.87	16.9	40.1	57.5
27	6.27	9.0	21.7	52.5	75.4
28	8.22	11.8	28.5	68.9	98.8
29	10.7	15.5	37.4	90.3	129.0
30	14.1	20.3	48.9	118.0	
31	18.5	26.6	64.2		
32	24.3	34.8	84.1		
33	31.8	45.6	110.0		
34	41.7	59.8	144.0		
35	54.6	78.4			
36	71.6	102.0			
37	93.9	134.0			
38	123.0				

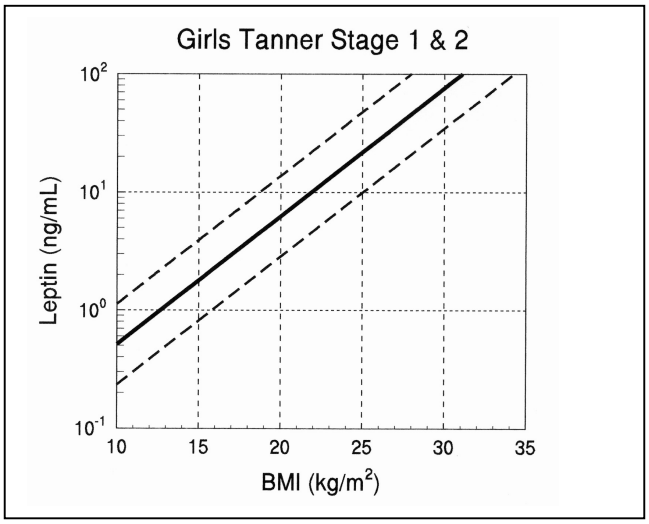

Fig. 2 Reference ranges for human serum concentrations as a function of BMI: Girls in Tanner stages 1 + 2 (see text).

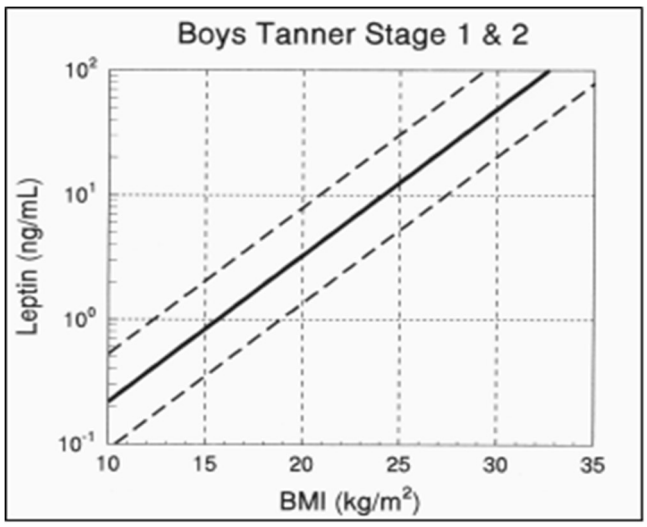

Fig. 3 Reference ranges for human serum concentrations as a function of BMI: Boys in Tanner stages 1 + 2 (see text).

Table 4 Girls in Tanner stages 3 and 4

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.32	0.41	0.73	1.29	1.63
12	0.41	0.52	0.92	1.63	2.06
13	0.52	0.66	1.16	2.07	2.61
14	0.65	0.83	1.47	2.61	3.31
15	0.83	1.05	1.87	3.31	4.19
16	1.05	1.33	2.36	4.19	5.30
17	1.33	1.68	2.99	5.30	6.71
18	1.68	2.13	3.78	6.71	8.49
19	2.13	2.69	4.79	8.5	10.8
20	2.69	3.41	6.06	10.7	13.6
21	3.41	4.31	7.67	13.61	17.2
22	4.32	5.46	9.71	17.2	21.8
23	5.46	6.91	12.3	21.8	27.6
24	6.91	8.75	15.6	27.6	34.9
25	8.75	11.1	19.7	34.9	44.2
26	11.1	14.0	24.9	44.2	56.0
27	14.0	17.7	31.6	56.0	70.9
28	17.8	22.5	39.9	70.9	89.7
29	22.5	28.4	50.5	89.7	114.0
30	28.4	36.0	63.9	114.0	144.0
31	36.0	45.6	80.9	144.0	
32	45.6	57.7	80.2	144.0	
33	57.7	73.0	102.0		
34	73.0	92.4	130.0		
35	92.4	117.0			
36	117.0	148.0			
37	148.0				

Table 5 Boys in Tanner stages 3 and 4

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.03	0.05	0.18	0.58	0.94
12	0.04	0.07	0.22	0.71	1.16
13	0.49	0.08	0.27	0.88	1.43
14	0.06	0.10	0.33	1.08	1.75
15	0.07	0.12	0.40	1.32	2.16
16	0.09	0.15	0.49	1.63	2.65
17	0.11	0.18	0.61	2.00	3.26
18	0.14	0.23	0.75	2.46	4.01
19	0.17	0.28	0.92	3.03	4.93
20	0.21	0.34	1.13	3.72	6.06
21	0.26	0.42	1.39	4.58	7.46
22	0.32	0.52	1.71	5.63	9.17
23	0.39	0.64	2.10	6.92	11.3
24	0.48	0.78	2.58	8.51	13.9
25	0.59	0.96	3.18	10.5	17.0
26	0.73	1.19	3.91	12.9	21.0
27	0.89	1.46	4.80	15.8	25.8
28	1.10	1.79	5.90	19.4	31.7
29	1.35	2.20	7.26	23.9	39.0
30	1.66	2.71	8.93	29.4	48.0
31	2.05	3.33	11.0	36.2	58.9
32	2.51	4.09	13.5	44.5	72.4
33	3.09	5.04	16.6	54.7	89.1
34	3.80	6.20	20.4	67.2	109.0
35	4.68	7.62	25.1	82.6	134.0
36	5.75	9.37	30.9	101.0	
37	7.07	11.5	37.9	124.0	
38	8.7	14.2	46.7		
39	10.7	17.4	57.4		
40	13.1	21.4	70.5		

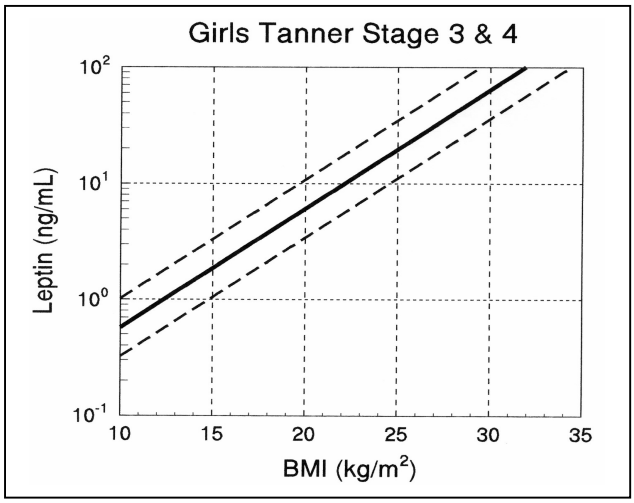

Fig. 4 Reference ranges for human serum concentrations as a function of BMI: Girls in Tanner stages 3 + 4 (see text).

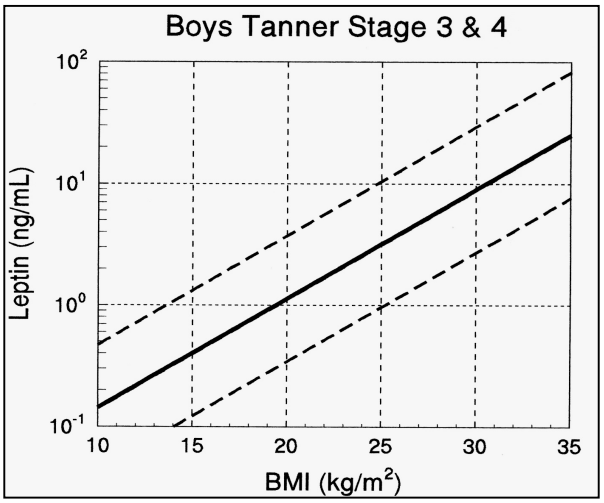

Fig. 5 Reference ranges for human serum concentrations as a function of BMI: Boys in Tanner stages 3 + 4 (see text).

Table 6 Girls in Tanner stage 5

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.50	0.66	1.34	2.71	3.62
12	0.58	0.77	1.56	3.15	4.21
13	0.67	0.89	1.81	3.67	4.89
14	0.78	1.04	2.11	4.26	5.69
15	0.91	1.21	2.45	4.96	6.62
16	1.05	1.41	2.85	5.76	7.70
17	1.22	1.64	3.31	6.70	8.95
18	1.42	1.90	3.85	7.79	10.4
19	1.66	2.21	4.48	9.06	12.1
20	1.93	2.57	5.20	10.5	14.1
21	2.24	2.99	6.05	12.3	16.4
22	2.60	3.48	7.03	14.2	19.0
23	3.03	4.04	8.18	16.6	22.1
24	3.52	4.70	9.51	19.3	25.7
25	4.09	5.46	11.0	22.4	29.9
26	4.76	6.35	12.9	26.0	34.8
27	5.53	7.39	15.0	30.3	40.4
28	6.43	8.59	17.39	35.2	47.0
29	7.48	9.99	20.2	40.9	54.7
30	8.70	11.6	23.5	47.6	63.5
31	10.1	13.5	27.3	55.3	73.9
32	11.8	15.7	31.8	64.4	85.9
33	13.7	18.3	37.0	74.9	99.9
34	15.9	21.2	43.0	87.0	116.0
35	18.5	24.7	50.0	101.0	135.0
36	21.5	28.7	58.1	118.0	
37	25.0	33.4	67.6	137.0	
38	29.1	38.8	78.6		
39	33.8	45.1	91.4		
40	39.4	52.5	106.0		

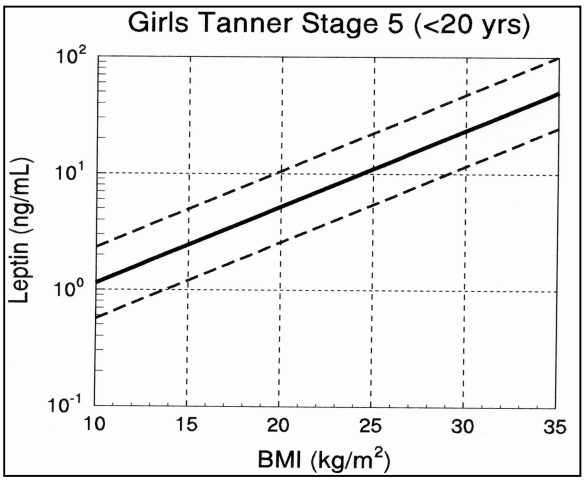

Fig. 6 Reference ranges for human serum concentrations as a function of BMI: Girls in Tanner stage 5 (see text).

Table 7 Boys in Tanner stage 5

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.03	0.05	0.16	0.47	0.73
12	0.04	0.06	0.18	0.54	0.84
13	0.05	0.07	0.21	0.62	0.97
14	0.05	0.08	0.24	0.72	1.12
15	0.06	0.10	0.28	0.84	1.30
16	0.07	0.11	0.33	0.97	1.51
17	0.08	0.13	0.38	1.12	1.74
18	0.1	0.15	0.44	1.3	2.02
19	0.11	0.17	0.51	1.50	2.34
20	0.13	0.2	0.59	1.74	2.7
21	0.15	0.23	0.68	2.01	3.13
22	0.17	0.27	0.79	2.33	3.62
23	0.20	0.31	0.91	2.69	4.19
24	0.23	0.36	1.05	3.12	4.85
25	0.27	0.41	1.22	3.61	5.62
26	0.31	0.48	1.41	4.17	6.5
27	0.36	0.55	1.63	4.83	7.52
28	0.41	0.64	1.89	5.59	8.71
29	0.48	0.74	2.19	6.47	10.1
30	0.55	0.86	2.54	7.49	11.7
31	0.64	1.00	2.94	8.67	13.5
32	0.74	1.15	3.4	10.0	15.6
33	0.86	1.33	3.94	11.6	18.1
34	0.99	1.54	4.55	13.4	20.9
35	1.15	1.79	5.27	15.6	24.2
36	1.33	2.07	6.10	18.0	28.1
37	1.54	2.39	7.06	20.8	32.5
38	1.78	2.77	8.17	24.1	37.6
39	2.06	3.21	9.46	27.9	43.5
40	2.38	3.71	10.9	32.3	50.3

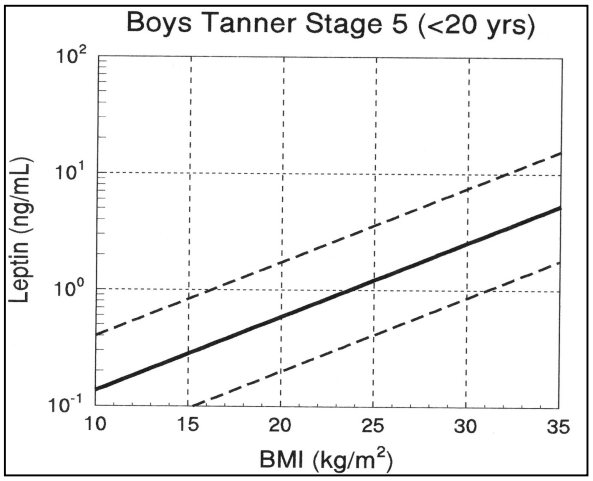

Fig. 7 Reference ranges for human serum concentrations as a function of BMI: Boys in Tanner stage 5 (see text).

Table 8 Women

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.46	0.65	1.53	3.59	5.10
12	0.53	0.75	1.77	4.16	5.90
13	0.61	0.87	2.05	4.82	6.83
14	0.71	1.01	2.37	5.58	7.91
15	7.82	1.17	2.75	6.46	9.17
16	0.95	1.35	3.18	7.48	10.61
17	1.10	1.57	3.68	8.66	12.3
18	1.28	1.81	4.27	10.0	14.2
19	1.48	2.10	4.94	11.6	16.5
20	1.71	2.43	5.72	13.4	19.1
21	1.99	2.82	6.62	15.6	22.1
22	2.30	3.26	7.67	18.0	25.6
23	2.66	3.78	8.88	20.9	29.3
24	3.08	4.38	10.3	24.2	34.3
25	3.57	5.07	11.9	28.0	39.7
26	4.13	5.87	13.8	32.4	46.0
27	4.79	6.79	16.0	37.5	53.3
28	5.54	7.87	18.5	43.5	61.7
29	6.42	9.11	21.4	50.4	71.5
30	7.43	10.6	24.8	58.3	82.8
31	8.61	12.2	28.7	67.5	95.8
32	9.97	14.1	33.3	78.2	111.0
33	11.5	16.4	38.5	90.5	129.0
34	13.4	19.0	44.6	105.0	149.0
35	15.5	22.0	51.6	121.0	
36	17.9	25.4	59.8	141.0	
37	20.8	29.5	69.3		
38	24.0	34.1	80.2		
39	27.8	39.5	92.9		
40	32.2	45.7	108.0		

Table 9 Men

Percentile (µg/L)					
BMI (kg/m²)	1	5	50	95	99
11	0.03	0.05	0.15	0.44	0.69
12	0.04	0.06	0.18	0.55	0.87
13	0.05	0.08	0.23	0.69	1.08
14	0.06	0.09	0.28	0.85	1.34
15	0.07	0.12	0.35	1.06	1.67
16	0.09	0.15	0.44	1.33	2.09
17	0.12	0.18	0.55	1.65	2.60
18	0.14	0.23	0.68	2.06	3.24
19	0.18	0.28	0.85	2.57	4.04
20	0.22	0.35	1.06	3.20	5.03
21	0.23	0.44	1.32	3.98	6.27
22	0.35	0.54	1.64	4.97	7.81
23	0.43	0.78	2.05	6.19	9.73
24	0.54	0.85	2.55	7.71	12.1
25	0.67	1.05	3.18	9.61	15.1
26	0.83	1.31	3.96	12.0	18.8
27	1.04	1.64	4.94	14.9	23.5
28	1.30	2.04	6.15	18.6	29.2
29	1.61	2.54	7.67	23.2	36.4
30	2.01	3.16	9.56	28.9	45.4
31	2.51	3.94	11.9	36.0	56.6
32	3.12	4.91	14.8	44.9	70.5
33	3.89	6.12	18.5	55.8	87.8
34	4.85	7.63	23.0	69.6	109.0
35	6.04	9.51	28.7	86.7	136.0
36	7.53	11.8	35.8	108.0	
37	9.38	14.8	44.6	135.0	
38	11.7	18.4	55.5		
39	14.6	22.9	69.2		
40	18.2	28.6	86.2		

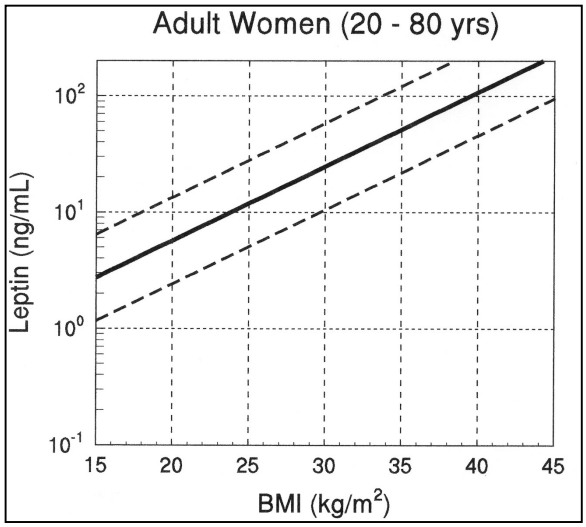

Fig. 8 Reference ranges for human serum concentrations as a function of BMI: Women (see text).

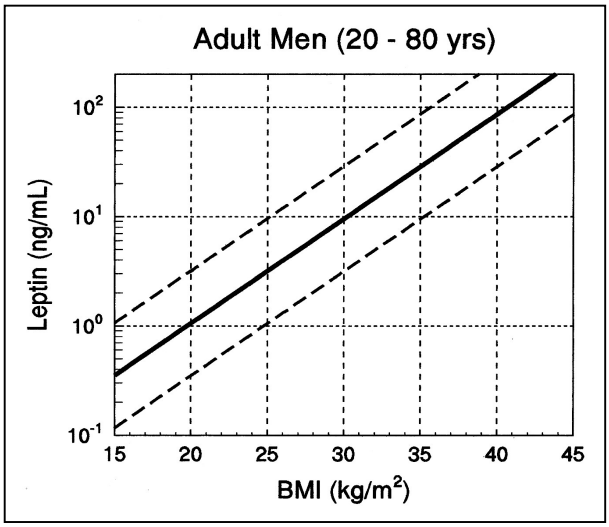

Fig. 9 Reference ranges for human serum concentrations as a function of BMI: Men (see text).

13 Assay properties and validation

13.1 Analytical sensitivity

The analytical sensitivity of the Leptin Sensitive ELISA E077 is 0.01 ng/mL (blank plus twice the standard deviation of the blank).

13.2 Recovery and precision

Recombinant leptin (WHO/NIBSC IS 97/594; 15,16) was added to Dilution Buffer **DIL** and serum samples. The leptin concentration of the samples enriched in this manner was measured and the relative recovery calculated. The results are shown in Table 10.

Table 10 Relative recovery of leptin WHO/NIBSC 97/594 in serum samples [%]

Leptin added [ng/mL]	5	10	15
Sample 1	109	108	108
Sample 2	108	104	109
Sample 3	105	102	85

13.3 Precision

The inter-assay and intra-assay coefficients of variation are both well below 10%. Representative determinations are shown in Table 11 and Table 12.

Table 11 Inter-assay variance

	Mean [ng/mL]	Standard deviation [ng/mL]	CV [%]
Sample 1	2.04	0.147	7.2
Sample 2	6.93	0.423	6.1
Sample 3	14.86	1.11	7.5

Table 12 Intra-assay variance

	Mean [ng/mL]	Standard deviation [ng/mL]	CV [%]
Sample 1	22.44	1.28	4.35
Sample 2	4.1	0.108	2.63

13.4 Linearity

Dilutions from 1:5 to 1:320 were tested with 4 serum samples. The measured leptin values are shown in Table 13.

Table 13 Dilutions of serum samples from 1:5 to 1:320

Dilution	Sample A [ng/mL]	Sample B [ng/mL]	Sample C [ng/mL]	Sample D [ng/mL]
1:5	10	26	-	23
1:8	10	25	39	25
1:10	11	26	36	26
1:20	11	28	37	28
1:40	11	29	39	29
1:80	12	29	41	30
1:160	10	30	40	31
1:320	11	27	37	30

13.5 Interference

Interference by haemoglobin, bilirubin and triglycerides in leptin measurement was tested by adding different amounts of these substances to human serum samples. The measured values in Table 14 demonstrate that neither bilirubin, triglycerides nor haemoglobin have any effect on the measurement of leptin in human serum at the maximum concentrations shown.

Table 14 Interferences. The relative quantities of leptin measured in a comparison of enriched serum and native serum are shown here in [%]

	Triglycerides 100 mg/mL	**Bilirubin 200 µg/mL**	**Haemoglobin 1 mg/mL**
Sample 1	95	101	94
Sample 2	107	105	105
Sample 3	111	101	101

14 References

1. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. 1994 Positional cloning of the mouse obese gene and its human homologue. Nature. 372:425-432.
2. Halaas JL, Gajiwala KS, Maffei M, et al. 1995 Weight-reducing effects of the plasma protein encoded by the obese gene. Science. 269:543-546.
3. MacDougald OA, Hwang CS, Fan H, Lane MD. 1995 Regulated expression of the obese gene product (leptin) in white adipose tissue and 3T3-L1 adipocytes. Proc Natl Acad Sci U S A. 92:9034-9037.
4. Rentsch J, Chiesi M. 1996 Regulation of ob gene mRNA levels in cultured adipocytes. FEBS Lett. 379:55-59.
5. Wabitsch M, Jensen PB, Blum WF, et al. 1996 Insulin and cortisol promote leptin production in cultured human fat cells. Diabetes. 45:1435-1438.
6. Stephens TW, Basinski M, Bristow PK, et al. 1995 The role of neuropeptide Y in the antiobesity action of the obese gene product. Nature. 377:530-532.
7. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. 1995 Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science. 269:546-549.
8. Pelleymounter MA, Cullen MJ, Baker MB, et al. 1995 Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 269:540-543.
9. Levin N, Nelson C, Gurney A, Vandlen R, de-Sauvage F. 1996 Decreased food intake does not completely account for adiposity reduction after ob protein infusion. Proc Natl Acad Sci U S A. 93:1726-1730.
10. Sinha MK, Ohannesian JP, Heiman ML, et al. 1996 Nocturnal rise of leptin in lean, obese, and non-insulin- dependent diabetes mellitus subjects. J Clin Invest. 97:1344-1347.
11. Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller W, Phinney SD. 1996 Gender difference in plasma leptin concentrations. Nature Med. 2:949-950.
12. Rosenbaum M, Nicolson M, Hirsch J, et al. 1996 Effects of gender, body composition, and menopause on plasma concentrations of leptin. J Clin Endocrinol Metab. 81:3424-3427.
13. Hassink SG, Sheslow DV, de Lancey E, Opentanova I, Considine RV, Caro JF 1996 Serum leptin in children with obesity: relationship to gender and development. Pediatrics. 98:201-203.
14. Blum WF, Juul A; Reference ranges of serum leptin,s.318-326. In:Leptin -the voice of adipose tissue, Blum WF et al, eds., Johann Ambrosius Verlag, Heidelberg, 1997.
15. Robinson CJ, Gaines-Das R,Woollacott D, et al. 2001 The first international standard for human leptin and the first international standard for mouse leptin: comparison of candidate preparations by in vitro bioassays and immunoassays. J Molecular Endocrinol. 27: 69-76.
16. Adresse NIBSC: Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, Great Britain; https://www.nibsc.org