Leptin Sensitive ELISA

Enzyme Immunoassay for Sensitive Quantitative Determination of

human Leptin

English

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

+2-8°C

96 wells

REF **E077**

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	Lot-Batch Number/ Charge-Chargennummer/ Lot-Code du lot/ Lotto-Numero di lotto/ Lote-Código de lote/ Lote-Código do Lote/Lot-Partijnumme/ Lot-Batchkode/ Partisatskod/ Numer serii/ Tétel-sarzs szám/ Číslo šarže/ Číslo šarže/ Партиден номер/Partii number/ Παρτίδα-αριθμός παρτίδας/ Lot-număr lot/ Številka serije/ Erä
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	Store at between/ Lagerung bei zwischen/ Conserver à entre/ Conservare a tra/ Conservar a temp. entre/ Armazenar entre/ Bewaar bij tussen/ Opbevares mellem/ Förvaras vid/ Przechowywać w/ Tárolási tartomány/ Skladujte v rozsahu / Skladujte v rozmezí/ Температурно ограничение/ Säilitada temperatuuridel/ Φύλαξη σε θερμοκρασία/ Depozitare între/ Skladiščenje med/ Säilytys x-y Celsiusasteen lämpötilassa
	Contains sufficient for x tests/ Inhalt ausreichend für x Tests/ Contient suffisant pour x tests/ Contenuto sufficiente per x test/ Contenido suficiente para x pruebas/ Conteúdo suficiente para x testes/ Bevat voldoende voor x bepalingen/ Indeholder tilstrækkeligt til x prøver/ Innehållet räcker till x analyser/ Zawartość na x testów/ Tartalma x teszt elvégzésére elegendő/ Obsahuje materiál pre x testov/ Obsah dostačuje pro x testů/ Съдържание достатъчно за x тестове/ Sisust jätkub x katse jaoks/ Το περιεχόμενο επαρκεί για x δοκιμές/ Conţinut suficient pentru x teste/ Vsebina zadostuje za x preizkusov/ Sisältö riittää x testille
	Incubation time/ Inkubationszeit/ Temps d'incubation/ Tempo d'incubazione/ Tiempo de incubación/ Tempo de incubação/ incubatietijd/ Inkubationstid/ inkubationstid/ Czas inkubacji/ Inkubációs idő/ Inkubačná lehota/ Inkubační doba/ Инкубационен период/ Inkubatsiooniaeg/ Χρόνος επώασης/ Timp de incubare/ Inkubacijska doba/ inkubaatioaika
	Incubate at/ Inkubation bei/ Incuber à/ Incubare a/ incubar a/ Incubar a/ incubatietemperatuur/ Inkubation ved/ inkubation vid/ Inkubacja przy/ Inkubáció hőmérséklete/ Inkubácia pri/ Inkubace při/ Инкубира се при/ Inkubatsioon temperatuuril/ Επώαση στους/ Incubare la/ Inkubacija pri/ Inkubaatiolämpötila
	Mix tubes with a Vortex mixer/ Mix Röhrchen mit Vortex Mixer/ Mélanger à l'aide d'un vortex/ Miscelare la provetta con agitatore Vortex/ Tubos de mezcla con mezclador de vórtex/ Misturar os tubos com um agitador Vortex/ buisjes mengen met een Vortex/ Blanderør med Vortex-mixer/ Blanda rören med en vortexblandare/ Miksowanie rurek w mikserze Vortex/ Csövecskék keverése örvénykeverővel/ Premiešať pomocou prístroja Vortex/ Promíchat pomocí přístroje Vortex/ Разбъркване на епруветките с миксер Vortex/ Segada torukesi Vortexi mikseriga/ Αναμίξτε τους σωληνίσκους με αναδευτήρα Vortex/ Amestecaţi eprubetele cu ajutorul unui agitator vortex/ Mešanje cevčic z mešalnikom Vortex/ Sekoita putket Vortex sekoittajalla
MTP	Miktrotiterplate/ Mikrotiterplatte/ plaque de microtitrage/ Piastra di microtitolazione/ Placa de microtitulación/ Placa de Microtitulação/ Microtiterplaat/ Mikrotiterplade/ mikrotiterplatta/ microtiterplaat/ Płytka microtiter/ Mikrotiter lap/ Mikrotitračná podložka/ Mikrotitrační podložka/ Микротитърна плака/ Mikrotiiterplaat/ Τρυβλίο μικροτιτλοδότησης/ Microplacă/ Mikrotitrska plošča/ Mikrotitrauslevy
Rec in	Reconstitute in/ Rekonstituieren in/ Reconstituer dans/ Ricostituire nel/ Reconstituir en/ Reconstituir em/ Reconstitueren in/ Rekonstituér i/ Rekonstituera/ Rekonstytuować w/ Helyreállítás/ Znovu pripraviť za/ Znovu připravit za/ Разтваряне в/ Moodustada uuesti/ Ανασυστήστε σε/ Reconstituire în/ Predelava v/ Rekonstituoi
SPE	Sample/ Probe/ Echantillon/ Campione/ Muestra/ Amostra/ Monster/ Prøve/ prov/ Próbka/ Minta/ Vzorka/ Vzorek/ Проба/ Proov/ Δείγμα/ Probă/ Vzorec/ Näyte
DET	Antibody and Enzyme Conjugate/ Antikörper- und Enzymkonjugat/ anticorps conjuguée et conjugué enzymatique/ Coniugato di anticorpo ed enzima/ Conjugado de anticuerpos y enzimas/ Conjugado Anticorpo-Enzima/ antilichaam- en enzymconjugaat/ Antistoffer og enzym-konjugat/ antikropps- och enzymkonjugat (antikropp och enzym, konjugat)/ Koniugat antyciał i enzymów/ Antitest és enzim páros/ Protilátkový a enzymatický konjugát/ Protilátkový a enzymatický konjugát/ Антитяло и ензим конюгат/ Antikehad ja ensüümi konjugaat/ Σύμπλοκο αντισώματος-ενζύμου/ Compuşi din anticorpi şi enzime/ Antitelesa in konjugat encima/ Vasta-aine ja entsymi konjugaatti
DIL	Dilution Buffer/ Verdünnungspuffer/ Tampon de dilution/ Tampone di diluizione/ Tampón de dilución/ / Tampão de diluição / Verdunningsbuffer/ / Fortyndingsbuffer/ Utspädningsbuffert / Bufor rozcieńczający/ / Hígító puffer/ Riediaci pufor/ Ředicí pufr / Буфер за разреждане/ Lahjenduspuhver/ Ρυθμιστικό διάλυμα αραίωσης / Tampon de diluare/ Pufer za redčenje/ Laimennuspuskuri

X:X	Dilute / Verdünnen / Diluer / Diluire / Diluir / Diluir / Verdunnen / Fortyndes / Späd / Rozcieńczanie / Hígítás / Riediť / Ředit / Разреждане / Lahjendada / Αραιώστε / Diluaţi / Razredčiti / Laimennetaan
CAL A-E	Calibrator X/ Kalibrator X/ calibrateur X/ calibratore X/ calibrador X/ calibrador X/ kalibrator X/ kalibrator X/ kalibrator X/ kalibrator X/ kalibrátor X/ kalibrátor X/ kalibrátor X/ калибратор X/ kalibraator X/ Βαθμονομητής X/ calibrator X/ kalibrator X/ kalibraattori X
CTR1 / CTR2	Control X/ Kontrolle X/ Contôle X/ controllo X/ control X/ Controle X/ controle X/ Kontrol X/ Kontroll X/ kontrolne X/ Ellenőrző X/ Kontrolné X/ Kontrolní X/ Контролен X/ Kontroll X/ ελέγχου X/ control X/ Kontrolni X/ Kontrolli X
WB	Washing Buffer Concentrate/ Waschpufferkonzentrat/ Tampon de lavage conc./ Tampone di lavaggio concentrato/ Tampón de lavado concentrado/ Tampão de Lavagem Concentrado/ wasbuffer, geconcentreerd/ Vaskebufferkoncentrat/ Vaskebufferkoncentrat/ tvättbuffertkoncentrat/ Bufor płukania koncentrat/ Mosópuffer koncentrátum/ Koncentrát vymývacieho pufra/ Концентрат на промивен буфер/ Pesupuhvri kontsentraat/ Συμπύκνωμα ρυθμιστικού διαλύματος έκπλυσης/ Concentrat pentru tamponul de spălare/ Koncentrat izpiralnega pufra/ Pesuliuositiiviste
WB 1:20	Washing Buffer/ Waschpuffer/ Tampon de lavage/ Tampone di lavaggio/ Tampón de lavado/Tampão de Lavagem/ wasbuffer/ Vaskebuffer/ tvättbuffert/ Bufor płukania/ Mosópuffer/ Vymývací pufer/ Vymývací pufr/ Промивен буфер/ Pesupuhver/ Ρυθμιστικό διάλυμα έκπλυσης/ Tampon pentru spălare /Izpiralni pufer/ Pesuliuos
S	Substrate/ Substrat/ Substrat/ Substrato/ Substrato/ Substrato/ substraat/ Substrat/ Substrat/ Substrat/ Szubsztrátum/ Substrát/ Substrát/ Субстрат Substraat/ Υπόστρωμα/ Substrat/ Substrat/ Substraattiliuos
STP	Stop Solution/ Stopplösung/ Stop Solution/ Soluzione di stop/ Stop Solución/ Solução Stop/ stopoplossing/ Stopopløsning/ Stopplösning/ Stop roztwór/ Megállító oldat/ Roztok na ukončenie/ Roztok pro ukončení/ Стопиращ разтвор/ Stopp-lahus/ Διάλυμα διακοπής/ Soluţie de oprire/ Stop raztopina/ Pysäytysliuos
TAPE	Cover Plate with sealing tape/ Platte abkleben/ Recouvrir la microplaque avec bande adhésive/ Coprire la piastra con nastro adesivo/ Cubrir la placa con una cinta adhesiva/ Cobrir a Placa com fita adesiva/ plaatje met tape afdekken/ Afdækningsplade med tape/ maskera platta/ Odkleić płytkę/ Tányér leragasztása/ Oblepiť podložku lepiacou páskou/ Olepit podložku lepicí páskou/ Плака с лента за запечатване/ Katta plaat isoleerkleeplindiga/ Κολλήστε το πλακίδιο με κολλητική ταινία/ Aoperiţi placa cu o bandă adezivă/ Prelepiti ploščo/ Peitä mikrotitrauslevy oheisella teipillä
MEASURE	Measure plate within 30 min at 450 nm (Referencefilter ≥590 nm)/ Ausmessung innerhalb von 30 min bei 450 nm (Referenzfilter ≥ 590 nm)/ Mesure lábsorbance en léspace de 30 min à 450 nm avec ≥590 nm longueur dónde pour référence/ Misurazione entro 30 min. a 450 nm (filtro di riferimento ≥ 590 nm)/ Medición de la placa dentro de los siguientes 30 min a 450 nm (filtro de referencia ≥ 590nm)/ Medir a placa dentro de 30 min a 450 nm (Filtro de referência ≥ 590 nm)/ Binnen 30 minuten bij 450 nm meten (referentiefilter ≥ 590 nm)./ Mål plade i løbet af 30 min ved nm (referencefilter ≥590 nm)/ Mät inom 30 min vid 450 nm (referensfilter ≥ 590 nm)./ Pomiar w ciągu 30 min przy 450 nm (filtr odniesienia ≥ 590 nm)/ Ki mérés 30 percen belül 450 nm-nél (referenciaszűrő ≥ 590 nm)/ Merať 30 minút pri 450 nm (Referenčných filtrov ≥590 nm)/ Měřit 30 minut při 450 nm (Referenční filtr ≥ 590 nm)/ Отчитане в рамките на 30 min при 450 nm (референтен филтър ≥ 590 nm)/ Mõõtmine 30 min jooksul 450 nm korral (võrdlusfilter ≥ 590 nm)/ Μέτρηση εντός 30 min στα 450 nm (φίλτρο αναφοράς ≥ 590 nm)/ Măsurare în decurs de 30 min la 450 nm (filtru de referinţă ≥ 590 nm)/ Izmerite ploščico v 30 min pri 450 nm (referenčni filter ≥590nm)/ Mittaa 30 minuutin aikana 450 nm:ssä (referenssi suodatin ≥ 590 nm)
Literature	Literature/ Literatur/ Bibliographie/ Letterario/ Bibliografía/ Literatura documentação/ literatuur/ Litteratur/ litteratur/ Literatura/ Irodalom/ Literatúra/ Literatura/ Литература// Kirjandus/ Βιβλιογραφία/ Bibliografie/ literatura/ Lähdeluettelo
International Test Description	International test description/ internationale Testanleitung/ description internationale de test/ Istruzioni per il test internazionali/ Descripción de ensayo internacional/ Descrição internacional do teste/ internationale testbeschrijving/ internationell testbeskrivning/ Opis testu międzynarodowego/ nemzetközi teszt-útmutató/ Medzinárodný návod k testu/ Mezinárodní návod k testu/ rahvusvaheline katse kirjeldus/ Διεθνείς οδηγίες για εργαστηριακές δοκιμές/ instrucţiuni internaţionale pentru testare/ mednarodna navodila za preizkus/ Kansainvälinen käyttöohje
End	in all required wells/ in allen benötigten Vertiefungen/ dans tous les godets requis/ in tutti i pozzetti richiesti/ en todos los pozos requeridos/ em todos os tubos necessários/ in alle nodige putjes/ i alle nødvendige brønde/ i alla nödvändiga brunnar/ we wszystkich potrzebnych wgłębieniach/ minden szükséges forrásban/ vo všetkých potrebných miestach/ ve všech potřebných místech/ във всички необходими ямки/ kõigis vajalikes süvendites/ σε όλες τις απαραίτητες κοιλότητες/ în toate cavităţile necesare/ v vseh zahtevanih vdolbinah/ kaikkiin tarvittaviin mikrotitrauslevyn syvennyyksiin

Read entire protocol before use!
For Research Use Only.
Not for use in diagnostic procedures.
CAUTION: Not for human or animal therapeutic or diagnostic use.
For in vitro use only.
For professional use only.

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Instructions for use

Leptin Sensitive ELISA E077	96 Determinations
Principle of the test	Enzyme-linked Immunoassay
Duration (incubation period)	1.75 h
Antibodies	monoclonal antibodies, ready for use
Buffer	Ready for use and 20fold concentrate
Calibrators	5 single Calibrators: 0.05 – 5 µg/L, lyophilized recombinant human Leptin
Reference material	International Standard WHO/ NIBSC 97/594, recombinant Leptin
Assay Range	0.01 – 50 µg/L
Control	2 Controls, freeze-dried
Sample	human serum / plasma, other human body fluids e.g. urine, saliva Precise measurement also in lean subjects
Required sample volume	25 µL
Sample dilution	1:10
Analytical sensitivity	ø 0.01 µg/L
Intra- / Interassay Variance	ø < 10 %
Exemplary 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, 1997

1 INTENDED USE

Measurement of human Leptin in human serum, plasma and in other human body fluids e.g. urine, saliva, breast milk, for research use only!

2 INTRODUCTION

Leptin, the product of the ob gene (1,2), is a recently discovered single-chain proteohormone with a molecular weight of 16 kD, which is thought to play a key role in the regulation of body weight. Its amino acid sequence exhibits no major homologies with other proteins (1). Leptin is almost exclusively produced by differentiated adipocytes (3-5). It acts on the central nervous system, in particular the hypothalamus, thereby suppressing food intake and stimulating energy expenditure (2,6-9). Leptin receptors - alternatively spliced forms exist that differ in length - belong to the cytokine class I receptor family (10-12). They are found ubiquitously in the body (10, 11, 13, 14) indicating a general role of leptin, which is currently not fully understood. A circulating form of the leptin receptor exists which acts as one of several leptin binding proteins (15). Besides its metabolic effects, leptin was shown to have a strong influence on a number of endocrine axes. In male mice, it blunted the starvation-induced marked decline of LH, testosterone, thyroxine and the increase of ACTH and corticosterone. In female mice, leptin prevented the starvation-induced delay in ovulation (16). Ob/ob mice, which are leptin deficient due to an ob gene mutation, are infertile. This defect could be corrected by administration of leptin, but not through weight loss due to fasting (17), suggesting that leptin is pivotal for reproductive functions.

All these actions may, at least in part, be explained by the suppressive effect of leptin on neuropeptide Y (NPY) expression and secretion by neurons in the arcuate nucleus (6,18,19). NPY is a strong stimulator of appetite (20,21) and is known to be involved in the regulation of

various pituitary hormones, e.g. suppression of GH through stimulation of somatostatin (22,23), suppression of gonadotropins (23) or stimulation of the pituitary-adrenal axis (21).

The most important variable that determines circulating leptin levels is body fat mass (24-26). Obviously, under conditions of regular eating cycles, leptin reflects the proportion of adipose tissue (27) showing an exponential relationship (37). This constitutive synthesis of leptin is modulated by a number of non-hormonal and hormonal variables. Stimulators in both rodents and humans are overfeeding (28,29), insulin (3,5,30-33) and glucocorticoids (5,34-36). Suppression has been shown for fasting (27), cAMP and beta-3-adrenoceptor agonists (35). From these findings it becomes clear that leptin is an integral component of various metabolic and endocrine feedback loops (38).

Serum leptin levels show a moderate **circadian variation** with a peak during the night at about 2 a.m. (37). The leptin values at this time are about 30 to 100 % higher than the levels measured in the morning or early afternoon. This variation together with the influence of food intake needs to be taken into account, when blood samples are collected.

This ELISA kit is suited for measuring human leptin in serum or plasma, other biological fluids (e.g. saliva, urine or cerebrospinal fluid), and conditioned adipocyte culture media for scientific purposes.

3 PRINCIPLE

The Mediagnost Sensitive ELISA for Leptin E077 is a so-called Sandwich-Assay using two specific and high affinity antibodies. The Leptin in the samples binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-Leptin-Antibody binds in turn to the immobilised Leptin. The second antibody is biotinylated and will be applied in a mixture with a Streptavidin-Peroxidase-Enzyme Conjugate. In the closing substrate reaction the turn of the colour will be catalysed quantitatively depending on the Leptin-level of the samples.

4 WARNINGS AND PRECAUTIONS

For research and professional use only 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. Mediagnost 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. A Material Safety Data sheet is 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 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.

Human Serum
Following components contain human serum: **Controls CTR1 and CTR2**
Source human serum for the Controls 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 specimens should be treated as potentially infectious.
Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step. Use separate pipette tips for each sample, control and reagent to avoid cross contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

Reagents CAL A-E, DET, DIL, WB
Contain as preservative a mixture of **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 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.

Stop Solution STP
The Stop 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.

4.1 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

Serum and plasma samples.

With this immunoassay kit, human leptin can also be determined for the measurement of leptin with low concentrations, e.g. in samples in body fluids other than serum (e.g. urine, liquor, breast milk) as well as in conditioned adipocyte culture media **for research use only**, for **scientific purposes**.

5.1 Blood Sample Types

Significant deviation of Leptin levels in corresponding Serum- or EDTA-Plasma samples were not found. If commercially available tubes for Citrate-plasma are used for preparation, samples will be diluted, resulting Leptin values will be therefore reduced.

5.2 Blood Samples

The blood samples for serum preparation should be gained according to standardized venipuncture procedure. Hemolytic reactions have to be avoided.
The human serum or plasma samples should be obtained in the morning or in the early afternoon (2:00 p.m.) if the nutritional status is normal.
Leptin levels show a circadian variation with a peak during the night at about 2 a.m. (37). This variation together with the influence of food intake needs to be taken into account, when blood samples are collected.

Required Serum/ Plasma sample volume: 25 µL

5.3 Serum- / Plasma- Sample stability

In firmly closable sample vials

- Storage at 20-25°C: 2 days
- Storage at -20° C: min. 2 years
- Freeze-thaw cycles max. 5

The storage of samples over a period of 2 years at -20°C, showed no influence on the reading. Freezing and thawing of samples should be minimized, 5 Freezing-Thawing showed no effect on samples.

5.4 Interference

Hemoglobin, triglyceride and bilirubin in the sample do not interfere to a concentration of **1 mg/mL**, **100 mg/mL** and **100 µg/mL.** However, the use of hemolytic, lipemic or icteric samples should be validated by the user.

5.5 Sample dilution

Samples have to be diluted in Dilution Buffer **DIL.**
For **Serum and Plasma** samples we recommend a dilution of 1:10.
<u>Suggestion for dilution protocol:</u>
Dilute e.g. 225 µL Dilution Buffer **DIL** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add 25 µL Serum-or Plasma (Dilution 1:10). After mixing use 2 x 100 µL from the dilution in the assay.
If Leptin levels over 50 ng/mL are expected, the sample should be diluted higher in Dilution Buffer **DIL**, e.g. 1:20.

The hLeptin concentrations may be **completely different in cell culture supernatants and body fluids of human origin other than serum**. The optimal dilution must be found out by the customer. E.g. in samples of urine or breast milk very low levels were determined. Furthermore also this kind of samples must be diluted at least 1:5. If samples are used undiluted, the reaction will be interfered and the values are reduced.

6 REAGENTS PROVIDED

6.1 Materials provided

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

MTP	**Microtiter plate,** ready for use, coated with mouse-anti-Leptin-antibody. Wells are separately breakable.	**(8x12) wells**
CAL A-E	**Calibrators**, lyophilized, (recombinant human Leptin), concentrations are given on vial labels and on quality certificate ng/mL.	**5 x 1 mL**
CTR1	**Control 1,** lyophilised, (human serum) concentration is given on quality certificate	**1 x 250 µL**
CTR2	**Control 2,** lyophilised, (human serum) concentration is given on quality certificate	**1 x 250 µL**
DET	**Antibody-HRP-Conjugate,** ready for use, mouse-anti-hLeptin-antibody biotinylated + streptavidin horseradish peroxidase conjugate	**1 x 12 mL**
DIL	**Dilution Buffer,** ready for use	**1 x 60 mL**
WB	**Washing Buffer,** 20-fold concentrated solution	**1 x 50 mL**
S	**Substrate,** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised tetramethylbencidine.	**1 x 12 mL**
STP	**Stop Solution,** ready for use, 0.2 M sulphuric acid.	**1 x 12 mL**
-	**Sealing Tape,** for covering the **microtiter plate.**	**2 x**
	**Instructions for use**	**1 x**
--	**Quality Certificate**	**1 x**

6.2 Materials required, but not provided

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

7 TECHNICAL NOTES

Storage Conditions

Store the kit at 2-8°C after receipt until its expiry date. The lyophilized reagents should be stored at –20 °C after reconstitution. 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:** Calibrators **A-E** and Controls **CTR1 and CTR2** 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 **WB** 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 Calibrators **A – E** and Controls **CTR1** and **CTR2** are reconstituted with the Dilution Buffer **DIL**. 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

The required volume of Washing Buffer **WB** is prepared by 1:20 dilution of the provided 20fold concentrate with Aqua dest.

After reconstitution dilute the Controls **CTR1 and CTR2** with the Dilution Buffer **DIL** in the same ratio (1:10) as the sample.

Assay Procedure

When performing the assay, Blank, Calibrators **A-E**, Controls **CTR1, CTR2** and the samples should be pipette as fast as possible. To avoid distortions due to differences in incubation times, Antibody-HRP-Conjugate **DET** as well as the succeeding Substrate **S** should be added to the plate in the same order and in the same time interval as the samples. Stop Solution **STP** should be added to the plate in the same order as Substrate **S**.
All determinations (Blank, Calibrators **A-E**, Controls **CTR1**, **CTR2** 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 **S**, stabilised Tetramethylbencidine, is photosensitive–storage 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 **WB** 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 ASSAY PROCEDURE

All determinations (Calibrators, Control **CTR1 & CTR2** and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.

When performing the assay, the Calibrators, Controls and the samples should be pipetted as fast as possible (e.g., <15 minutes).

All incubations have to be conducted at room temperature (20-25°C)!

To avoid distortions due to differences in incubation times, Antibody-POD-Conjugate **DET** as well as the following Substrate **S** should be added to the plate in the same order and in the same time interval as the samples. Stop Solution **STP** should be added to the plate in the same order as the Substrate **S**.

1) Add **100 µL** Dilution Buffer **DIL** to the first wells (blank). Subsequently, add 100 µL of each Calibrator or diluted Control (CTR1&CTR2) or diluted Sample to the following wells.

2) Cover the wells with sealing tape and incubate the plate for **1 hour** shaking with **350 rpm**.

3) After incubation aspirate the contents of the wells into a disinfectant (possible theoretically risk of infection!) and wash the wells **3 times** with **300 µL** of **Washing Buffer WB** / well respectively. The washing buffer WB should incubate at least for 15 seconds/cycle

4) Pipette **100 µL** of the **Antibody-HRP-Conjugate DET** in each well and incubate **30 minutes** shaking with **350 rpm**.

5) After incubation wash the wells 3 times with Washing Buffer WB as described in step 3

6) Pipette **100 µL of the Substrate S** in each well.

7) Incubate the plate for **15 minutes in the dark at room temperature (20 - 25°C)**.

8) Stop the reaction by adding **100 µL of Stop Solution STP.**

9) Measure the colour reaction within 30 minutes at **450 nm** (reference filter ≥**590 nm**).

9 CALCULATION OF RESULTS

For the evaluation of the assay it is required that the absorbance values of the blank should be below 0.25, and the absorbance of Calibrator E should be above 1.00. Samples, which yield higher absorbance values than Calibrator E, should be re-tested with a dilution.

9.1 Establishing of the calibration curve

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

1) Calculate the **mean absorbance** value for the blank from the duplicated determination (well A1/A2).

2) Subtract the mean absorbance of the blank from the mean absorbances of all other samples, controls and calibrators.

3) Plot the calibrator concentrations on the x-axis versus the mean value of the absorbance of the calibrators on the y-axis.

4) Recommendation: Calculation of the calibration 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 of the diluted controls **CTR1** and **CTR2** and diluted **samples** is obtained from the calibration curve. The **concentration** of the undiluted samples / controls in **ng/mL** can be calculated **by multiplication** with the respective dilution factor.

9.2 Example of a typical Calibration Curve

The following Calibration Curve is for demonstration only and cannot be used in place of data generation at the time of assay.

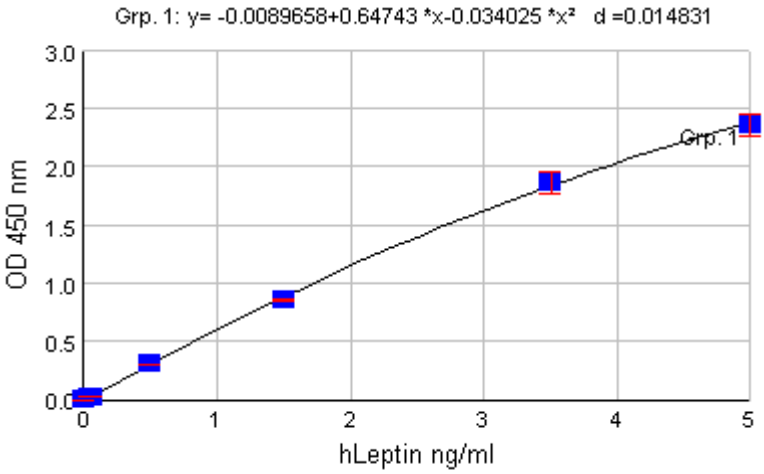

Figure 1: Exemplary Standard Curve with a polynomial 2rd degree as curve fit.

Exemplary calculation of the leptin concentration of the 1:10 diluted sample:

Measured extinction of your sample	0.293
Measured extinction of the blank	0.015

Your **software program** will calculate the leptin concentration of the diluted sample automatically by using the difference of sample and blank (0.278) for the calculation. You only have to determine the most suitable curve fit (here: polynomial 2nd degree).

In this exemplary case the following equation is solved by the program to calculate the Leptin concentration in the sample:

$$0.278 = -0.0089658+0{,}64743x -0.034025x^2$$
$$0.4524 = x$$

if the dilution factor (1:10) is taken into account the leptin concentration of the undiluted sample is

$$0.4524 \text{ ng/mL} \times 10 = 4.4524 \text{ ng/mL}$$

10 EXEMPLARY VALUES

Serum leptin levels are mainly determined by body fat mass with low levels in lean individuals and high levels in obese subjects. In addition, there is a clear gender difference with higher levels in females at a given percentage body fat. Further, leptin levels are influenced by pubertal development. Any attempt, therefore, to give ranges of expected leptin levels must account for these relationships.

The following exemplary serum leptin levels were referred to BMI as the major confounding independent variable and were stratified according to gender and pubertal development (45; see figures 2-9 and tables 2-9). After the age of 20 years, no significant age dependence was observed. These. The best-fit regression lines for the various subgroups are exponential curves of the form:

$$Leptin = a \cdot e^{(b \cdot BMI)}$$

Table 1 Constants a, b, c and d for calculation of leptin ranges and leptin SDS based on BMI. Groups of normal healthy individuals were stratified according to gender and pubertal stage/age. TS= Tanner stage, n= number of subjects, a,b,c and d = constants as defined in the text (see Chapter Expected Normal Values).

Cohort	n	a	b	c	d
Males:					
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
Females					
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 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.		
33	53.2	73.4			
34	68.4	94.3			
35	87.8	121.			
36	113				
37	145				

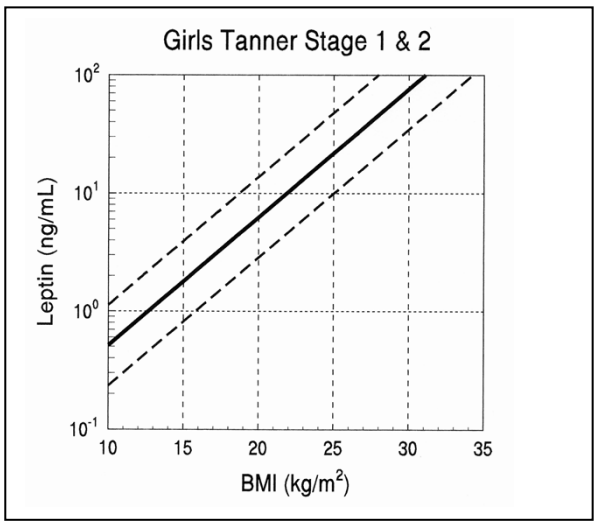

Figure 2 Reference ranges of human serum levels referring to BMI: Girls Tanner stage 1 & 2 (see text for details)

Table 3 Boys Tanner stages 1 and 2

Percentile (µg/L)					
BMI	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				

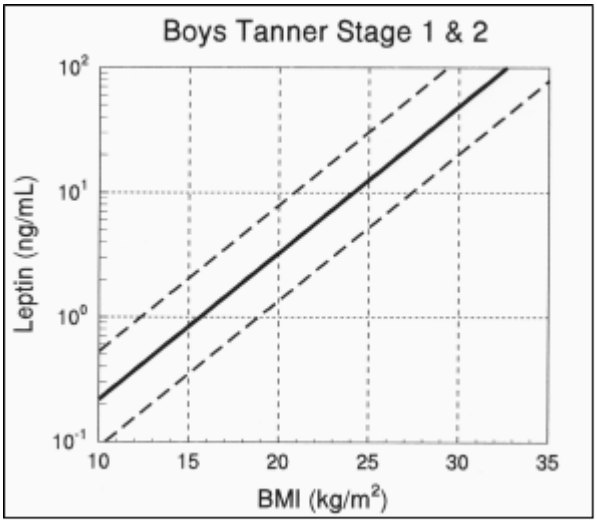

Figure 3 Reference ranges of human serum levels referring to BMI: Boys Tanner stage 1 & 2 (see text for details)

Table 4 Girls 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 Tanner stage 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.	124.0	
38	8.7	14.2	46.7		
39	10.7	17.4	57.4		
40	13.1	21.4	70.5		

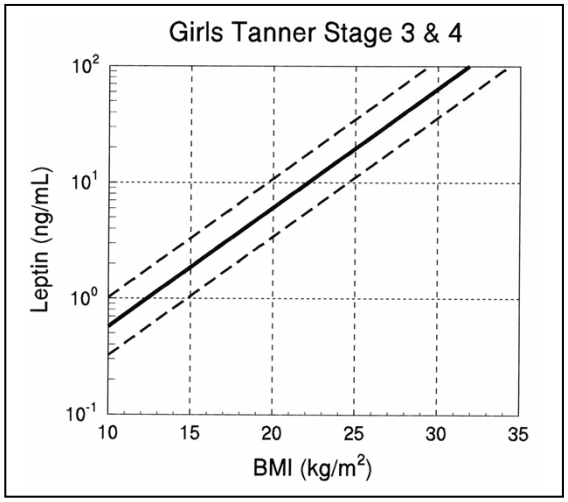

Figure 4 Reference ranges of human serum levels referring to BMI: Girls Tanner stage 3 & 4 (see text for details).

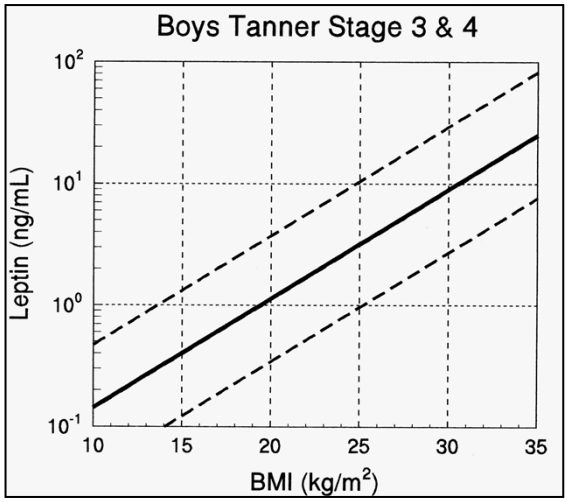

Figure 5 Reference ranges of human serum levels referring to BMI: Boys Tanner stage 3 & 4 (see text for details).

Table 6 Girls 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		

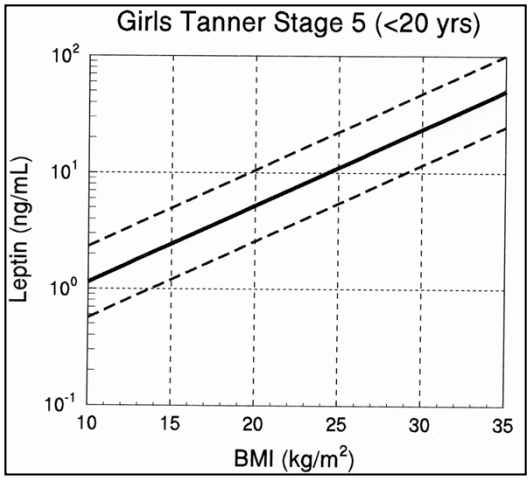

Figure 6 Reference ranges of human serum levels referring to BMI: Girls Tanner stage 5 (see text for details)

Table 7 Boys 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

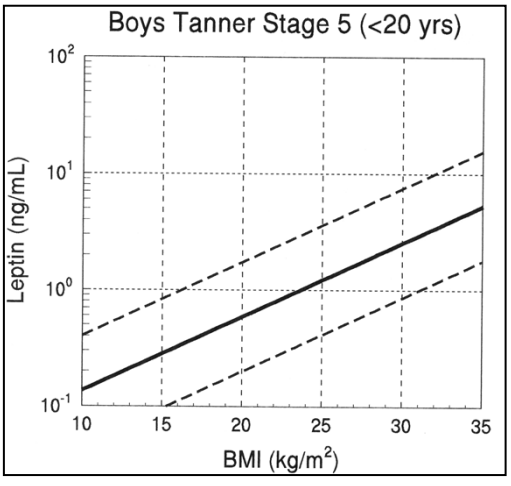

Figure 7 Reference ranges of human serum levels referring to BMI: Boys Tanner stage 5 (see text for details).

Table 8 Adult 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		

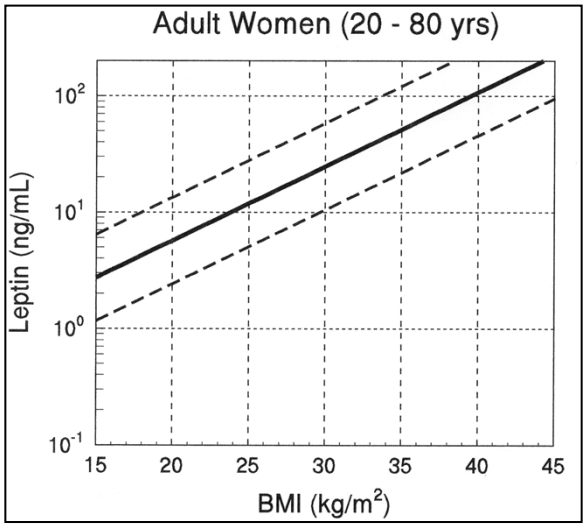

Figure 8 Reference ranges of human serum levels referring to BMI: Adult women (see text for details)

Table 9 Adult 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		

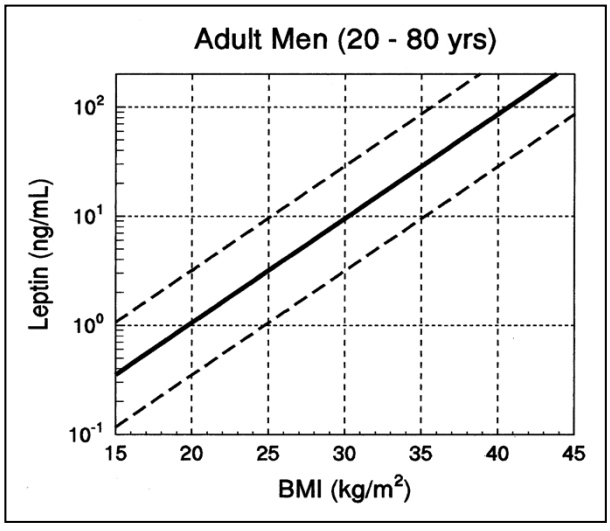

Figure 9 Reference ranges of human serum levels referring to BMI: Adult men (see text for details).

11 PERFORMANCE CHARACTERISTICS

11.1 Sensitivity

The analytical sensitivity of the assay yields 0.01 ng/mL (2x SD of zero standards in 12-fold determinations).

11.2 Precision

Intra- and Inter-Assay Variance

The inter- and intra assay coefficients of variability are below 10%. Exemplary determinations are shown in table 10 and 11.

Table 10 Inter-Assay-Variation

	Mean value (ng/mL)	Standard Deviation (ng/mL)	VC (%)
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 11 Intra-Assay-Variation

	Mean value (ng/mL)	Standard Deviation (ng/mL)	VC (%)
Sample 1	**22.44**	**1.28**	**4.35**
Sample 2	**4.1**	**0.108**	**2.63**

11.3 Linearity

Dilutions of 1:5 up to 1:320 have been tested with four serum samples and the amount of Leptin measured is shown in table 12. The data show that dilutions of 1:5 to 1:320 seem to give reasonable results (no deviation of more than 20% of the mean value of all dilutions tested). A dilution of 1:10 of serum samples is recommended in general.

Table 12 Dilution linearity of 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

11.4 Recovery

Recombinant Leptin (NIBSC) was added to Dilution Buffer (DIL) and serum samples. The leptin content of the so enriched samples was measured and relative recovery was calculated. Results are shown in table 13.

Table 13 Relative recovery of NIBSC leptin in serum samples [%]

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

11.5 Interference

Interference of bilirubin, hemoglobin and triglycerides was tested by adding different amounts of these substances to human serum containing Leptin. For comparison the same amount of buffer without any substance was also added to the serum. Table 14 demonstrates that neither bilirubin nor triglycerides or haemoglobin exert any influence on the measurement of Leptin in human serum.

Table 14 Interference of physiological substances

	Triglyceride 100 mg/mL	Bilirubin 100 µg/mL	Hemoglobin 1 mg/mL
Serum 1	95	101	94
Serum 2	107	105	105
Serum 3	111	101	101

11.6 Species Cross-Reactivity

The cross-reactivities of several commercially available animal sera were tested in this assay. For this purpose, the sera in various dilutions were used as a sample in the Mediagnost Leptin ELISA E077.
NO SIGNAL was detected in the sera of the following species

Horse, cattle, chicken, rabbit, dog, guinea pig, sheep, mouse, goat, donkey, rat, cat, pig.

12 COMPARISON STUDIES

The correlation between ELISA E077 and the Mediagnost LEP-R40 radioimmunoassay is shown in Figure 10.

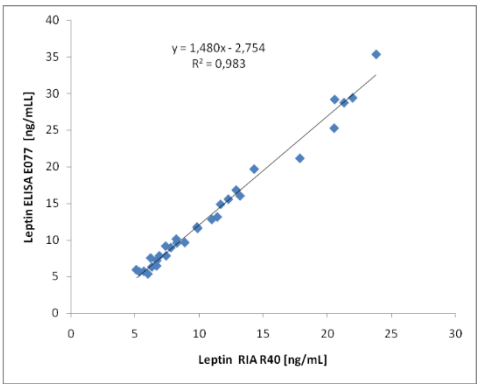

Figure 10 Assay comparison
– Mediagnost Radioimmunoassay LEP-R40 and ELISA E077

13 LITERATURE

LITERATURE

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14 International Assay Description

International Test Description

CAL A-E	**Rec in 1 mL DIL**	**-**
CTR1	**Rec in 250 µL DIL**	**1:10 DIL ➔ CTR1 1:10**
CTR2	**Rec in 250 µL DIL**	**1:10 DIL ➔ CTR2 1:10**
WB	**-**	**1:20 A. dest. ➔ WB 1:20**
SPE	**-**	**1:10 DIL ➔ SPE 1:10**

°C 20-25°C

100 µL	**DIL**	A1/A2
100 µL	**CAL A (0.05 ng/mL)**	B1/B2
100 µL	**CAL B (0.5 ng/mL)**	C1/C2
100 µL	**CAL C (1.5 ng/mL)**	D1/D2
100 µL	**CAL D (3.5 ng/mL)**	E1/E2
100 µL	**CAL E (5 ng/mL)**	F1/F2
100 µL	**CTR1 1:10**	G1/G2
100 µL	**CTR2 1:10**	H1/H2
100 µL	**SPE 1:10**	
TAPE		
1 h °C 20-25°C ↔ 350 rpm		
3x 300 µL	**3x WB 1:20**	
100 µL	**DET**	
TAPE		
0.5 h °C 20-25°C ↔ 350 rpm		
3x 300 µL	**3x WB 1:20**	
100 µL	**S**	
0.25 h °C 20-25°C		
STP		
MEASURE		

15 ASSAY PROCEDURE

Preparation of reagents		Reconstitution	Dilution
CAL A-E	**Calibrators**	in **1 mL** Dilution Buffer **DIL**	-
CTR1 **CTR2**	**Controls**	in **250 µL** Dilution Buffer **DIL**	**1:10** in Dilution Buffer **DIL** ➔ **CTR1 1:10** ➔ **CTR2 1:10**
WB	**Washing Buffer** 20-fold conc.	-	**1:20** with **Aqua dest.** ➔ **WB 1:20**
Sample dilution in **Dilution Buffer DIL**, e.g. 1:10 ➔ **SPE 1:10**			
Before assay procedure bring all reagents to room temperature **20-25°C**.			

Assay Procedure in Double Determination:

Pipette	Reagents	Position
100 µL	Dilution Buffer **DIL** (**Blank**)	A1/A2
100 µL	Calibrator **A** (**0.05 ng/mL**)	B1/B2
100 µL	Calibrator **B** (**0.5 ng/mL**)	C1/C2
100 µL	Calibrator **C** (**1.5 ng/mL**)	D1/D2
100 µL	Calibrator **D** (**3.5 ng/mL**)	E1/E2
100 µL	Calibrator **E** (**5 ng/mL**)	F1/F2
100 µL	Control **CTR1 1:10**	G1/G2
100 µL	Control **CTR2 1:10**	H1/H2
100 µL	Sample **SPE 1:10**	in the rest of the wells according the requirements
Cover the wells with the sealing tape.		
Sample Incubation: 1 h at 20-25°C, 350 rpm		
3 x 300 µL	Aspirate the contents of the wells and **wash** 3 x with 300 µL each Washing Buffer **WB 1:20** **/ well**	In each well
100 µL	Antibody-HRP-Conjugate **DET**	In each well
Cover the wells with the sealing tape.		
Incubation: 30 Minutes at 20-25°C, 350 rpm		
3 x 300 µL	Aspirate the contents of the wells and **wash** 3 x with 300 µL each Washing Buffer **WB 1:20** **/ well**	In each well
100 µL	Substrate **S**	In each well
Incubation: 15 Minutes in the Dark at 20-25°C		
100 µL	Stop Solution **STP**	In each well
Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength.		