m/rLeptin ELISA

Enzyme Immunoassay for Quantitative Determination of

Mouse and Rat Leptin (Obese Protein)

English

All countries:
For Research Use Only. Not for use in diagnostic procedures.

2-8°C X 96 wells

h **E06**

Distributed By:
IBL-America, Inc.
8201 Central Ave NE, Suite P
Minneapolis, MN 55432, USA
info@ibl-america.com
(888) 523 1246

mediagnost®
Gesellschaft für Forschung und Herstellung von Diagnostika GmbH

M**:** Aspenhaustr. 25 • D-72770 Reutlingen / Germany
Telefon: + 49 - (0) 7121 51484-0 • Fax: + 49 - (0) 7121 51484-10
E-Mail: contact@mediagnost.de • http://www.mediagnost.de

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g	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
M	Manufactured by/ Hergestellt von/ Fabriqué par/ Prodotto da/ Fabricado por/ Fabricado por/ Vervaardigd doo/ Fabrikation af/ Tillverkad av/ Wyprodukowane przez/ Gyártotta/ Vyrobené/ Vyrobeno v/ Производител/ Tootja/ Κατασκευάζεται από/ Produs de/ Proizvajalec/ Valmistaja
h	Catalogue Number/ Bestellnummer/ Numéro de référence/ Numero di riferimento/ Número de referencia/ Número de Referência/ Referentienummer/ Referencenummer/ Beställningsnummer/ Numer katalogowy/ Rendelési szám/ Katalógové číslo/ Objednací číslo/ Каталожен номер/ Tellimisnumber/ Αρ. παραγγελίας/Număr comandă/ Številka naročila/ Viite tai tilausnumero
	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
X	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
W	Keep away from sunlight/ Nicht dem Sonnenlicht aussetzen/ Conserver à l´abri de la lumière/ Conservare al riparo della luce solare/ No exponer a la luz solar/ Proteger da luz solar/ Niet aan zonlicht blootstellen/ Må ikke udsættes for sollys/ Utsätt inte för solljus/ Nie wystawiać na słońce/ Napfénytől távol tartandó/ Nevystavovať slnečnému svetlu/ Nevystavovat sluwnečnímu světlu/ Да се предпазва от слънчева светлина/ Kaitsta otsese päikesekiirguse eest/ Κρατήστε το μακριά από την ηλιακή ακτινοβολία/ Ţineţi departe de lumina soarelui/ Ne izpostavljajte sončni svetlobi/ suojaa auringonvalolta
À	Incubation time/ Inkubationszeit/ Temps d'incubation/ Tempo d'incubazione/ Tiempo de incubación/ Tempo de incubação/ incubatietijd/ Inkubationstid/ Czas inkubacji/ Inkubációs idő/ Inkubačná lehota/ Inkubační doba/ Инкубационен период/ Inkubatsiooniaeg/ Χρόνος επώασης/ Timp de incubare/ Inkubacijska doba/ inkubaatioaika
°C	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
Ab	Antibody Conjugate/ Antikörperkonjugat/ Anticorps conjuguée/ Coniugato di anticorpo/ Conjugado de anticuerpos/ Conjugado anticorpo/ Antilichaamconjugaat/ Antistoffer-konjugat/ Antikroppskonjugat/ Koniugat antyciał/ Antitest páros/ Protilátkový konjugát/ Protilátkový konjugát/ Антитяло конюгат/ Antikehad konjugaat/ Σύμπλοκο αντισώματος/ Compuşi din anticorpi/ Antitelesa konjugat/ Vasta-aine konjugaatti

CONJ	Enzyme Conjugate/ Enzymkonjugat/ Conjugué enzymatique/ Coniugato di enzima/ Conjugado de enzimas/ Conjugado Enzima/ Enzymconjugaat/ Enzym-konjugat/ Enzymkonjugat/ Koniugat enzymów/ Enzim páros/ Enzymatický konjugát/ Enzymatický konjugát/ ензим конюгат/ Ensüümi konjugaat/ Σύμπλοκο –ενζύμου/ Compuşi din enzime/ Encima konjugat/ Enzymi-konjugaatti
BUF	Buffer/ Puffer/ Tampon/ Tampone/ Tampón/ Tampão/ Buffer/ Buffer/ Buffert/ Bufor/ Puffer/ Pufer/ Pufr/ Буфер/ Puhver/ Ρυθμιστικό διάλυμα/ Tampon/ Pufer/ Puskuri
DILU **BUF** X	Dilute in Buffer X/ Verdünnen in Puffer X/ Diluer dans le tampon X/ Diluire nel tampone X/ Diluir en tampón X/ Diluir no Tampão X/ Verdunnen in buffer X/ Fortyndes i buffer X/ Späd i buffert X/ Rozcieńczanie w buforze X/ Hígítás X pufferben/ Riediť v pufri X/ Ředit v pufru X/ Разреждане в буфер X/ Lahjendada puhvris X/ Αραιώστε σε ρυθμιστικό διάλυμα X/ Diluaţi în tamponul X/ Razredčiti v pufru X/ Laimennetaan x puskuriin
STD	Standard X/ Standard X/ Etalon X/ Standard X/ Estándar X/ Standard X/ Standaard X/ Standard X/ standard X/ Standard X/ Standard X/ Štandard X/ Standard X/ Стандарт X/ Standard X/ Πρότυπο X/ Standard X/ Standardni X/ Standardi X
Control	Control Serum X/ Kontrollserum X/ Contôle sérique X/ Siero di controllo X/ Suero de control X/ Soro de Controlo X/ controleserum X/ Kontrolserum X/ Kontrollserum X/ Serum kontrolne X/ Ellenőrző szérum X/ Kontrolné serum X/ Kontrolní serum X/ Контролен серум X/ Kontrollseerum X/ Ορός ελέγχου X/ Ser de control X/ Kontrolni serum X/ Kontrolli seerumi X
WASHBUF 20x	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
WASHBUF	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
SUBST **TMB**	Substrate/ Substrat/ Substrat/ Substrato/ Substrato/ Substrato/ substraat/ Substrat/ Substrat/ Substrat/ Szubsztrátum/ Substrát/ Substrát/ Субстрат Substraat/ Υπόστρωμα/ Substrat/ Substrat/ Substraattiliuos
H_2SO_4	Stopping 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)
Literatur	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

ENGLISH mouse/rat Leptin ELISA INSTRUCTIONS FOR USE

TABLE OF CONTENTS

m/rLeptin ELISA	96 Determinations
RUO	For Research Use Only
Principle of the test	Enzyme Immunoassay
Duration (incubation period)	3 h
Antibody	100fold concentrated
Enzyme Conjugate	100fold concentrated
Dilution Buffer	Ready for use
Washing Buffer	20fach Konzentrat
Standard	Recombinant Mouse Leptin, 7 Separate Standards: 25-1600 pg/ml, lyophilised
Calibration	Calibrated against the international Mouse-Leptin Standard, WHO (NIBSC Code 97/626)
Assay Range	10 pg/mL – 8000 pg/mL
Control	1 Control serum, lyophilised
Sample	Mouse- and Rat- Serum and Plasma
Required sample volume	Up to 50 µL max.
Sample dilution	At least 1:2
Sensitivity	10 pg/mL
Intra- / Interassay Variance	< 10 %

1 INTENDED USE

This enzyme immunoassay kit is suited for measuring Leptin in mouse and rat serum for scientific purposes.

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), high fat diets (41), 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).

3 PRINCIPLE

The Mediagnost m/rLeptin ELISA, E06 is a so-called sandwich-assay. It utilizes two different specific high affinity polyclonal antibodies for this protein. The Leptin in the samples binds quantitatively to the immobilized antibody. In the following step, the biotinylated antibody in turn binds Leptin. After washing, a streptavidin-peroxidase-enzyme conjugate will be added, which will bind highly specific to the biotin of the antibody. Subsequently, the peroxidase catalyzes an enzymatic reaction resulting in a blue coloration. The intensity of the blue color depends on the Leptin content of the sample. The reaction is stopped by the addition of stop solution and color intensity is quantified by measuring the absorption.

4 WARNINGS AND PRECAUTIONS

For In Vitro Use only. For Professional use only.
The Mediagnost kit is suitable only for in vitro and not for internal use in humans and animals. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. 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.

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

Caution: This kit contains material of 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.

Reagents A-G, AK, EK, VP, WP
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 Solution (S)
The TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbencidine (<0.05%)

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

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

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

4.1 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

5.1 Sample type

Mouse and Rat Serum Plasma
Serum as well as Heparin-, EDTA- or Citrate-Plasma plasma are suitable samples. Possible dilution of the sample by the anticoagulant must be considered.

5.2 Specimen collection

Haemolytic reactions have to be avoided.

5.3 Required sample volume:

According to Leptin level of the sample, maximal 50 µL per test.
Samples should be diluted prior to measurement with Dilution Buffer **VP** depending on the expected values.

5.4 Sample stability

Undiluted serum specimen may be stored frozen at -20°C without loss of mouse/rat leptin. Repeated thawing and freezing should be avoided, although levels were found to be unaffected by a few cycles. Diluted samples are stable maximum of 2 h.

5.5 Sample dilution

Generally, a sample dilution of 1:5 to 1:20 is suitable. Depending upon species, stem and breeding and/or the individual experimental conditions this can, however, vary. If very low leptin concentrations are expected, 1:2 diluted samples might be used instead. However, if sample volume is limited, higher dilutions might be useful (provided that leptin concentration is sufficient).

- **1:5** with Dilution Buffer **VP**. E.g. for one double determination, pipette **200 µL** Dilution Buffer **VP** in PE-/PP-Tube (application of a multi-stepper is recommended in larger series); add **50 µL sample** (dilution 1:5). After mixing use **2 x 100 µL** of this dilution in the assay.
- Or, pipette **80 µL** Dilution Buffer **VP** in a well and add **20 µL** Serum (mix well). Depending on the expected Leptin values the samples can be diluted higher in Dilution Buffer **VP**.

6 MATERIALS

6.1 Materials provided

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

MTP	**Microtiter plate,** ready for use, coated with goat anti-mouse/rat Leptin-antibody. Wells are	**(8x12) wells**
A-G	**Standards**, lyophilized, (recombinant mouse Leptin), concentrations are given on vial labels and on quality	**7 x 1 mL**
KS	**Control Serum,** lyophilised, (Mouse serum), concentration is given on quality certificate.	**1 x 200 µL**
AK	**Antibody Conjugate,** 100-fold concentrated, contains goat biotinylated anti-mouse/rat leptin antibody**.**	**1 x 120 µL**
EK	**Enzyme Conjugate,** 100-fold concentrated contains HRP (Horseradish-Peroxidase)-labelled	**1 x 120 µL**
VP	**Dilution Buffer,** ready for use	**1 x 120 mL**
WP	**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**
SL	**Stopping Solution,** ready for use, 0.2 M sulphuric acid.	**1 x 12 mL**
-	**Sealing Tape,** for covering the **microtiter plate.**	**3 x**
	**Instructions for use**	**1 x**
--	**Quality Certificate**	**1 x**

6.2 Materials required, but not provided

- Distilled (Aqua destillata) or deionized water for dilution of the Washing Buffer **WP (A. dest.), 950 mL.**
- Precision pipettes and multichannel pipettes with disposable plastic tips
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Vortex-mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)
- Micro plate reader ("ELISA-Reader") with filter for 450 and [3] 590 nm

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. Reconstituted components should be stored at -20°C (or colder). For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Freezing is only possible once! The 1:20 diluted Washing Buffer **WP** is 4 weeks stable at 2-8°C

Preparation of reagents

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

Reconstitution

The Standards **A – G** and Control **KS** are reconstituted with the Dilution Buffer **VP**. It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam should result) with a Vortex mixer. Thaw quickly but gently for further use (no temperature increases above room temperature and no excessive vortexing). Freezing is only possible once! The wash buffer WP, diluted 1:20, is stable for 4 weeks.

Dilution

After reconstitution dilute the Control **KS** with the Dilution Buffer **VP** in the same ratio (1:5) as the sample.
The required volume of Washing Buffer **WP** is prepared by 1:20 dilution of the provided 20-fold concentrate with Aqua dest.

Assay Procedure

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

Incubation

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

Shaking

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

Washing

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

The danger of handling with potentially infectious material must be taken into account. **Manual washing** should be used. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. Decant contents into a biohazard bin, then blot plate on absorbent tissue. Wash the plate by adding 300µL Washing Buffer WP/well, then decant and blot on absorbent tissue. Repeat this step 2 more times for total of 3 washes.

8 ASSAY PROCEDURE

Preparation of reagents		Reconstitution:	Dilution
A-G	**Standards**	in **1 mL** Dilution Buffer **VP**	-
AK	**Antibody**		**1:100** with Dilution Buffer **VP**
EK	**Enzyme Conjugate**		**1:100** with Dilution Buffer **VP**
KS	**Control Serum**	in **200 µL** Dilution Buffer **VP**	**e.g. 1:5** with Dilution Buffer **VP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**
Dilute samples 1:5 in Dilution Buffer VP (Mix 50 µL Serum with 200 µL Dilution Buffer VP), mix immediately.			
Before assay procedure bring all reagents to room temperature **20-25°C**.			

Assay procedure in double determination		
Pipette	**Reagents**	**Position**
100 µL	Dilution Buffer **VP**	A1/A2
100 µL	Standard **A** (**25 pg/mL**)	B1/B2
100 µL	Standard **B (50 pg/mL)**	C1/C2
100 µL	Standard **C (100 pg/mL)**	D1/D2
100 µL	Standard **D** (**200 pg/mL)**	E1/E2
100 µL	Standard **E (400 pg/mL)**	F1/F2
100 µL	Standard **F** (**800 pg/mL)**	G1/G2
100 µL	Standard **G** (**1600 pg/mL)**	H1/H2
100 µL	Control Serum **KS** (1:5 diluted)	A3/A4
100 µL	Sample (1:5 diluted)	in the rest of the wells according
Cover the wells with the sealing tape.		
Sample-Incubation: 1 h at 20-25°C, 350 rpm		
3x 300 µL	Decant the contents of the wells and **wash** 3 x with 300 µL	In each well
100 µL	1:100 diluted Antibody Conjugate	In each well
Cover the wells with the sealing tape.		
Incubation: 1 h at 20-25°C, 350 rpm		
3x 300 µL	Decant the contents of the wells and **wash** 3 x with 300 µL	In each well
100 µL	1:100 diluted Enzyme Conjugate EK	In each well
Incubation: 30 Minutes at 20-25°C, 350 rpm		
3x 300 µL	Decant the contents of the wells and **wash** 3 x with 300 µL	In each well
100 µL	Substrate Solution **S**	In each well
Cover the wells with the sealing tape.		
Incubation: 30 Minutes in the Dark at 20-25°C		
100 µL	Stopping Solution **SL**	In each well
Measure the absorbance within **15 min** at **450 nm** with **≥ 590 nm** as reference wavelength.		

9 CALIBRATION OF THE ASSAY

The Mediagnost Mouse/Rat Leptin ELISA E06 has been calibrated against the International Reference Standard for mouse leptin. The definition of this international reference material, code 97/626, was evaluated in an international collaborative study, with Mediagnost test kits and with participation of Mediagnost (39). The standard preparation of the WHO with code 97/626 (39) is available from the NIBSC (40).

One ampoule of the preparation, reconstituted in 1 ml solution, will be quantified with this kit E06 to the nominal content of 4000 ng mouse leptin.

10 EVALUATION OF RESULTS

The absorbance values of the blank should be below 0.25, these of standard G (1600 pg/ml) should be above 1.0.
The determined and calculated concentration of Control KS should be within the range of the concentration given on vial label.

10.1 Establishing of the standard curve

The standards provided contain the following concentrations of recombinant mouse leptin:

Standard	**A**	**B**	**C**	**D**	**E**	**F**	**G**
pg/mL	**25**	**50**	**100**	**200**	**400**	**800**	**1600**

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 values.
3) Plot the standard concentrations on the x-axis versus the mean value of the absorbance of the standards on the y-axis.
4) Recommendation: Calculation of the standard curve should be done by using a computer program because the curve is in general (without respective transformation) not ideally described by linear regression. **A higher-grade polynomial,** or **four parametric logistic (4-PL) curve fit** or **non-linear regression** are usually suitable for the evaluation (as might be spline or point-to-point alignment in individual cases).
5) The Leptin concentration in pg/ml of the samples can be calculated by **multiplication** with **the respective dilution factor**.

10.2 Example of a typical standard curve

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

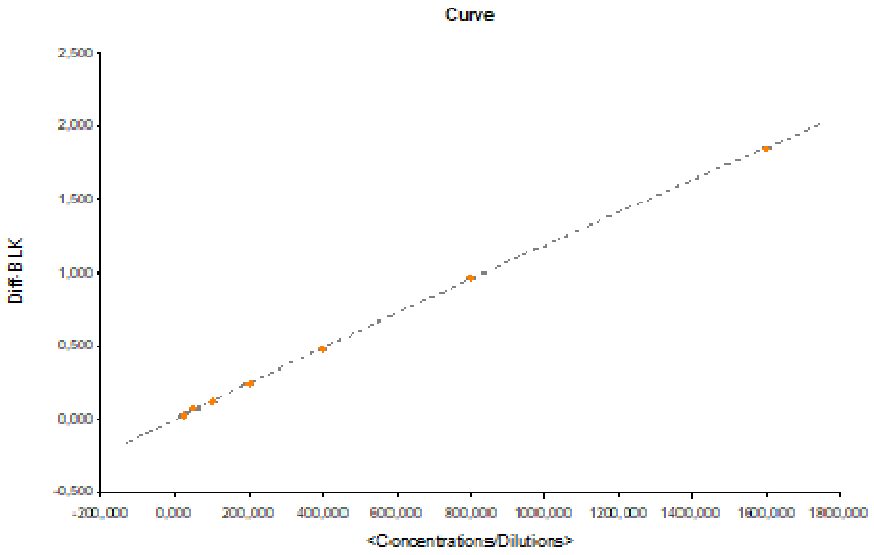

Figure 1 Examplary standard curve

	A	B	C	D	E	F	G
pg/mL	**25**	**50**	**100**	**200**	**400**	**800**	**1600**
OD **(450-620 nm) blank corrected**	0.031	0.072	0.116	0.244	0.483	0.968	1.841

The exemplary shown standard curve in Figure 3 **cannot** be used for calculation of your test results. You have to establish a standard curve for each test you conduct!

10.3 Exemplary calculation of Leptin concentrations

Sample Dilution: 1:5

Measured extinction of your sample:	0.794
Measured extinction of the blank	0.134
Difference Data of the sample	0.660

Your measurement program will calculate the Leptin concentration of the diluted sample automatically by using the difference of sample and blank for the calculation. You only have to determine the most suitable curve fit.

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

$$y = 0.00117x$$

$$x = 548.2 \text{ pg/mL}$$

If the dilution factor (**1:5**) is taken into account the Leptin concentration of the undiluted sample is

548.2 pg/mL ´ 5 = 2741 pg/mL

11 PERFORMANCE CHARACTERISTICS

The ELISA for Mouse/Rat Leptin E06 utilizes two specific high affinity polyclonal antibodies for these proteins. It recognizes quantitatively mouse leptin. Standards are prepared of recombinant mouse leptin.

A certain degree of cross reactivity against rat leptin allows to use the kit also for measuring rat leptin. Dilutions of rat samples were found as linear as mouse samples. Preparations of recombinant mouse and rat leptin from the same producer were compared regarding their quantification with this kit. The relative potency of the rat material was found to be approx. 25%, compared to the respective mouse material, and, based on the nominal declaration of the producer.

When working with rat samples, individual own calibrating of the kit values is recommended. E06 is calibrated against the WHO NIBSC mouse leptin standard code 97/626 (see above).

The cross reactivity against human leptin is 0.7%.

11.1 Sensitivity

The practical sensitivity of the assay is 10 pg/ml, i.e., 1 pg/well (calculated by extrapolation of the standard curve).

11.2 Precision

Intra- and Inter Assay Variance

Inter-assay and intra-assay variation coefficients were found to be < 10 %,

Table 1 Inter-Assay-Variation: Different sera, independently diluted, measured in duplicate

Sample 1 (pg/ml)	867.9	802.8	874.1	822.5	904.5	821.8	901.9
Sample 2 (pg/ml)	1208.2	1169.6	1306.5	1275.6	1276.8	1212.0	1246.5
Sample 3 (pg/ml)	627.9	631.2	601.0	638.7	590.2	612.2	586.3

Table 2 Intra-Assay-Variation: Different sera independently 1:5 diluted, measured 6fold each in duplicate

Sample 1 (pg/ml)	1262	1266	1197	1260	1218	1247
Sample 2 (pg/ml)	869	829	790	826	790	821
Sample 3 (pg/ml)	436	457	464	454	423	418

11.3 Linearity

Dilution was found to be linear over the standard range.

Table 3 Linearity of the sample dilution: Independent assays, independent dilutions as indicated, determinations in duplicate

Dilution	Serum 1 (pg/ml)	Serum 2 (pg/ml)	Serum 3 (pg/ml)	WHO NIBSC Code 97/626 (pg/ml)		
				nominal	1. Dilution results	2. Dilution results
1:2	1002	628	927	1500	1417.5	1609.3
1:4	1170	631	892	750	722.5	838.0
1:8	1212	601	855	375	343.6	397.3
1:16	1307	n.d.	n.d.	187.5	171.0	197.6
1:32	1424	n.d.	n.d.	93.75	88.4	98.2
1:64	1432	n.d.	n.d.	46.88	42.9	53.3
				23.44	21.0	n.d.

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13 INTERNATIONAL ASSAY DESCRIPTION

A-G	STD	Rec in 1 mL BUF VP	-
AK	AK		1:100 DILU BUF VP
EK	EK		1:100 DILU BUF VP
KS	Control	Rec in 200 µL BUF VP	1:5 DILU BUF VP
WP	WASHBUF	-	1:20 DILU A. dest.
-	SPE		1:5 DILU BUF VP
-	°C 20-25 °C ; À «	max 350 rpm	

100 µL	BUF VP	A1/A2
100 µl	STD A (25 pg/ml)	B1/B2
100 µl	STD B (50 pg/ml)	C1/C2
100 µl	STD C (100 pg/ml)	D1/D2
100 µl	STD D (200 pg/ml)	E1/E2
100 µl	STD E (400 pg/ml)	F1/F2
100 µl	STD F (800 pg/ml)	G1/G2
100 µl	STD G (1600 pg/ml)	H1/H2
100 µl	CONTROL KS 1:5 DILU BUF VP	A3/A4
100 µl	SPE 1:5 DILU BUF VP	End
	TAPE	
	À 1 h °C 20-25 « 350 rpm	
3x 300 µL	3x WASHBUF WP	
100 µL	CONJ AK	
	TAPE	
	À 1 h °C 20-25 « 350 rpm	
3x 300 µL	3x WASHBUF WP	
100 µL	CONJ EK	
	TAPE	
	À 30 min °C 20-25 « 350 rpm	
3x 300 µL	3x WASHBUF WP	
100 µL	SUBST TMB S	
	TAPE	
	À 30 min °C 20-25 ✳	
	H_2SO_4 SL	
	MEASURE	

14 ASSAY DESCRIPTION

Preparation of reagents		**Reconstitution:**	**Dilution**
A-G	**Standards**	in **1 mL** Dilution Buffer **VP**	-
AK	**Antibody Conjugate**		**1:100** with Dilution Buffer **VP**
EK	**Enzyme Conjugate**		**1:100** with Dilution Buffer **VP**
KS	**Control Serum**	in **200 µL** Dilution Buffer	**e.g. 1:5** with Dilution Buffer **VP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**
Dilute samples e.g. 1:5 in Dilution Buffer VP (Mix 50 µl Serum with 200 µL Dilution Buffer VP), mix immediately.			
Before assay procedure bring all reagents to room temperature **20-25°C**.			

Assay procedure in double determination

Pipette	**Reagents**	**Position**
100 µL	Dilution Buffer **VP (Blanc)**	A1/A2
100 µL	Standard **A** (**25 pg/mL**)	B1/B2
100 µL	Standard **B (50 pg/mL)**	C1/C2
100 µL	Standard **C (100 pg/mL)**	D1/D2
100 µL	Standard **D** (**200 pg/mL)**	E1/E2
100 µL	Standard **E (400 pg/mL)**	F1/F2
100 µL	Standard **F** (**800 pg/mL)**	G1/G2
100 µL	Standard **G** (**1600 pg/mL)**	H1/H2
100 µL	Control Serum **KS** (1:5 diluted)	A3/A4
100 µL	Sample (1:5 diluted)	in the rest of the wells according
Cover the wells with the sealing tape.		
Sample-Incubation: 1 h at 20-25°C, 350 **rpm**		
3x 300 µL	Decant the contents of the wells and **wash** 3 x with 300 µL	In each well
100 µL	1:100 diluted Antibody Conjugate	In each well
Cover the wells with the sealing tape.		
Incubation: 1 h at 20-25°C, 350 rpm		
3x 300 µL	Decant the contents of the wells and **wash** 3 x with 300 µL	In each well
100 µL	1:100 diluted Enzyme Conjugate EK	In each well
Incubation: 30 min at 20-25°C, 350 rpm		
3x 300 µL	Decant the contents of the wells and **wash** 3 x with 300 µL	In each well
100 µL	Substrate Solution **S**	In each well
Cover the wells with the sealing tape.		
Incubation: 30 Minutes in the Dark at 20-25°C		
100 µL	Stopping Solution **SL**	In each well
Measure the absorbance within **15 min** at **450 nm** with **≥ 590 nm** as reference wavelength.		