Manufactured for Immuno-Biological Laboratories Inc.
(IBL-America)
8201 Central Avenue, NE, Suite P
Minneapolis, MN 55432
Tel: 763-780-2955
Toll Free: 1-888-523-1246

sLEP-R ELISA

Enzyme Immunoassay for the Quantitative Determination of Human

Soluble Leptin Receptor (sLEP-R)

English

For research use only
Not for use in diagnostic procedures.
for professional use!

2-8°C X 96 wells

REF **R07**

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

Manufactured by Mediagnost / Germany
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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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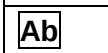
	Store at between/ Lagerung bei zwischen/ Conserver à entre/ Conservare a tra/ Conservar a temp. entre/ Armazenar entre/ Bewaar bij tussen/ Opbevares mellem/ Förvaras vid/ Przechowywać w/ Tárolási tartomány/ Skladujte v rozsahu / Skladujte v rozmezí/ Температурно ограничение/ Säilitada temperatuuridel/ 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
	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 slunečnímu 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/ suoja auringonvalolta
	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
	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
	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
	Sample/ Probe/ Echantillon/ Campione/ Muestra/ Amostra/ Monster/ Prøve/ prov/ Próbka/ Minta/ Vzorka/ Vzorek/ Проба/ Proov/ Δείγμα/ Probă/ Vzorec/ Näyte
	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 Instructions for use

sLEP-R ELISA, R07	96 Determinations
Regulatory Status	For Research Use Only. Not for diagnostic purposes.
Principle of the test	Enzyme Immunoassay
Duration (Incubation time)	5 h
Antibodies	Specific high-affinity antibodies
Dilution Buffer	Ready for use
Washing Buffer	20fold concentrate
Standard	8 single standards: 0 - 30 ng/mL, lyophilized
Assay Range	0.0385 – 150 ng/mL
Controls	2 serum controls, low and high level, respectively, lyophilized
Samples	human Serum or Plasma (EDTA, Heparin)
Required sample volume	30 µL single determination / 50 µL double determination
Sample dilution	1:5
analytical Sensitivity	Ø < 0.0385 ng/mL
Intra- / Inter-Assay Variance	Ø < 10 %
Linearity	1:5 – 1:90, Linear Regression $R^2 > 0.9$
Interference: Hemoglobin / Triglyceride / Bilirubin	No influence up to concentrations of: 1 mg/mL / 100 mg/mL / 100 µg/mL
Calibration	Recombinant eukaryotic expressed soluble leptin receptor (Mediagnost, #P48357 AA20-839+HIS tag)
Interference of rec. Leptin	No influence up to concentrations of 256 ng/mL
Expectation values	Healthy adult blood donors (male 19 / female 20)

1 INTENDED USE

Quantitative measurement of soluble human leptin receptor (sLEP-R) in human serum or plasma for research purposes.

2 INTRODUCTION

The adipokine leptin realizes signal transduction via four different leptin receptor (LEP-R) isoforms. The amount of functionally active LEP-R, however, is affected by constitutive shedding of the extracellular domain. The product of the cleavage process, the so-called soluble leptin receptor (sLEP-R, soluble Leptin receptor), is the main binding protein for leptin in human blood and modulates its bioavailability. Concentrations of sLEP-R are differentially regulated in metabolic disorders, such as type 1 diabetes mellitus or obesity and can therefore enhance or reduce leptin sensitivity. Lipotoxicity and apoptosis increase LEP-R cleavage via ADAM10-dependent mechanisms. In contrast, although increased sLEP-R concentrations seem directly to inhibit leptin effects, reduced amounts of sLEP-R may reflect decreased membrane expression of LEP-R. These findings, in part, explain alterations of leptin sensitivity that are associated with changes in serum sLEP-R concentrations seen in metabolic disorders (1-5).

3 ASSAY PRINCIPLE

The Mediagnost ELISA for Soluble Leptin Receptor (sLEP-R) is a so-called Sandwich-Assay using two specific and highly affine antibodies. The sLEP-R in the samples binds to the first antibody coated on the microtiter plate. In the following step the second specific anti-sLEP-R-antibody binds in turn to the immobilised sLEP-R. This is biotinylated and allows the binding of

a streptavidin-peroxidase enzyme conjugate. Subsequently, the peroxidase catalyzes an enzymatic reaction resulting in a blue coloration. The intensity of the blue color depends on the sLEP-R content of the sample. The reaction is stopped by the addition of stop solution. After stopping the reaction, the color intensity (then yellow) is quantified by measuring the absorbance and converted to the sLEP-R concentration using a standard curve.

4 WARNINGS AND PRECAUTIONS

For research and professional use only.

The Mediagnost **sLEP-R ELISA** kit is suitable only for in vitro use and not for internal use in humans and animals. Follow strictly the test protocol. Use the valid version of the package insert provided with the kit. Be sure that everything has been understood. 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. Safety Data Sheet available on request.

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

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

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

4.1 Human Serum

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

4.2 Reagents

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

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

Substrate Solution (S)
The TMB-Substrate (S) contains 3.3´,5.5´ Tetramethylbencidine (<0.05 %)

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

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

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

4.3 General first aid procedures:

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

5 SAMPLES

5.1 Sample Type

Serum and Plasma

The measurement of corresponding Serum and Heparin or EDTA plasma samples gives comparable results, no influence of the anticoagulant was detectable.

5.2 Specimen collections

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

5.3 Required sample volume

30 µL single determination / 50 µL double determination

5.4 Sample dilution

- **Dilution: e.g. 1:5** with Dilution Buffer **VP**
- **Example for a double determination:** Add **50 µL** Sample to **200 µL** Dilution Buffer **VP** (Dilution factor 5)
- **Minimum required sample dilution 1:5**

5.5 Sample stability

In firmly closable sample vials:

- Storage at Room Temperature 20-25°C: max. 2 days
- Storage at 4°C: max. 3 days
- Storage at -20°C: 2 years
- Freeze/Thaw cycles: max. 3

Freeze-Thaw cycles should be minimized. Up to 3 cycles showed no effect on the measured sLEP-R concentration.

5.6 Interference

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

6 MATERIALS

6.1 Materials Provided

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

MTP	**Microtiter plate,** ready for use, coated with human anti hLeptin-R antibody. Wells are separately breakable.	**12x8 wells**
A-H	**hLeptin Receptor Standards (rec.)**, lyophilized, concentrations are given on vial labels and on the QC-certificate.	**8 x 1 mL**
KS1	**Control Serum 1,** lyophilised, (human serum), concentration is given on the QC-certificate.	**1 x 500 µL**
KS2	**Control Serum 2,** lyophilized, (human serum), concentration is given on the QC-certificate.	**1 x 500 µL**
AK	**Antibody Conjugate,** ready for use, contains biotinylated anti-hLeptin-R-antibody**.**	**1 x 12 mL**
EK	**Enzyme Conjugate,** ready for use. contains Streptavidin-Peroxidase Conjugate**.**	**1 x 12 mL**
VP	**Dilution Buffer,** ready for use, **Please shake before use!**	**1 x 60 mL**
WP	**Washing Buffer,** 20-fold concentrated solution	**1 x 50 mL**
S	**Substrate,** ready for use, horseradish-peroxidase-(HRP) substrate, stabilised Tetramethylbencidine.	**1 x 12 mL**
SL	**Stopping Solution,** ready for use, 0.2 M sulphuric acid.	**1 x 12 mL**
-	**Sealing Tape,** for covering the **microtiter plate.**	**3 x**
	**Instructions for use**	**1 x**
--	**Quality Control Certificate**	**1 x**

6.2 Materials required, but not provided

- Distilled (Aqua destillata, A. dest.) or deionized water for dilution of the Washing Buffer **WP**, 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)
- Microplate 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.

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

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

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

Dilution
After reconstitution dilute the Control Sera **KS1** and **KS2** with the Dilution Buffer **VP** in the same ratio (1:5) as the sample.
The required volume of Washing Buffer **WP** is prepared by 1:20 dilution of the provided 20fold concentrate with Aqua dest.

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

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

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

Washing
Proper washing is of basic **importance** for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities e.g. high Standard A value, 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 is recommended. 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.

When using an **automatic microtiter plate washer**, the respective instructions for 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.

8 SUMMARY OF THE ASSAY PROCEDURE R07

Preparation of reagents		Reconstitution:	Dilution
A-H	**Standards**	in **1 mL** Dilution Buffer **VP**	-
KS1	**Control Serum 1**	in **500 µL** Dilution Buffer **VP**	**1:5** with Dilution Buffer **VP**
KS2	**Control Serum 2**	in **500 µL** Dilution Buffer **VP**	**1:5** with Dilution Buffer **VP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**

Sample dilution: with Dilution Buffer **VP 1:5. Don´t use samples undiluted!**

Before assay procedure bring all reagents to room temperature **20-25°C**.

Assay Procedure in Double Determination:

Pipette	Reagents	Position
100 µL	Standard **A (0 ng/mL**)	A1/A2
100 µL	Standard **B (0.625 ng/mL**)	B1/B2
100 µL	Standard **C (1.25 ng/mL**)	C1/C2
100 µL	Standard **D (2.5 ng/mL**)	D1/D2
100 µL	Standard **E** (**5.0 ng/mL**)	E1/E2
100 µL	Standard **F (10 ng/mL**)	F1/F2
100 µL	Standard **G (20 ng/mL**)	G1/G2
100 µL	Standard **H (30 ng/mL**)	H1/H2
100 µL	Control Serum **KS 1** (1:5 diluted)	A3/A4
100 µL	Control Serum **KS 2** (1:5 diluted)	B3/B4
100 µL	Sample (1:5 diluted)	in the rest of the wells according the requirements
Cover the wells with the sealing tape.		
Sample Incubation: 2 hours at 20-25°C, 350 rpm		
5 x 300 µL	Aspirate the contents of the wells and wash **5 x** with **300 µL** each Washing Buffer **WP/ well.**	In each well
100 µL	Antibody Conjugate **AK**	In each well
Cover the wells with the sealing tape.		
Incubation: 2 hours at 20-25°C, 350 rpm		
5 x 300 µL	Aspirate the contents of the wells and wash **5 x** with **300 µL** each Washing Buffer **WP/ well.**	In each well
100 µL	Enzyme Conjugate **EK**	In each well
Cover the wells with the sealing tape.		
Incubation: 30 minutes at 20-25°C, 350 rpm		
5 x 300 µL	Aspirate the contents of the wells and wash **5 x** with **300 µL** each Washing Buffer **WP/ well.**	In each well
100 µL	Substrate Solution **S**	In each well
Incubation: 30 minutes in the Dark at 20-25°C		
100 µL	Stopping Solution **SL**	In each well
	Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength.	

9 QUALITY CONTROL

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

9.1 Quality Criteria

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

10 EVALUATION OF RESULTS

10.1 Establishing the Standard Curve

The standards provided contain the following concentrations of sLEP-R :

Standard	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
ng/mL	0	0.625	1.25	2.5	5.0	10	20	30

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

10.2 Example of a Typical Standard Curve

The exemplary data and the standard curve in Figure 1 cannot be used for the calculation of the test results. You have to establish a standard curve for each test you conduct.

Table 1 Data which describe a typical standard curve.

Standard	A	B	C	D	E	F	G	H
ng/mL	0	0.625	1.25	2.5	5.0	10	20	30
$OD_{450\text{-}620\ nm}$	0.00	0.047	0.102	0.198	0.392	0.823	1.577	2.117

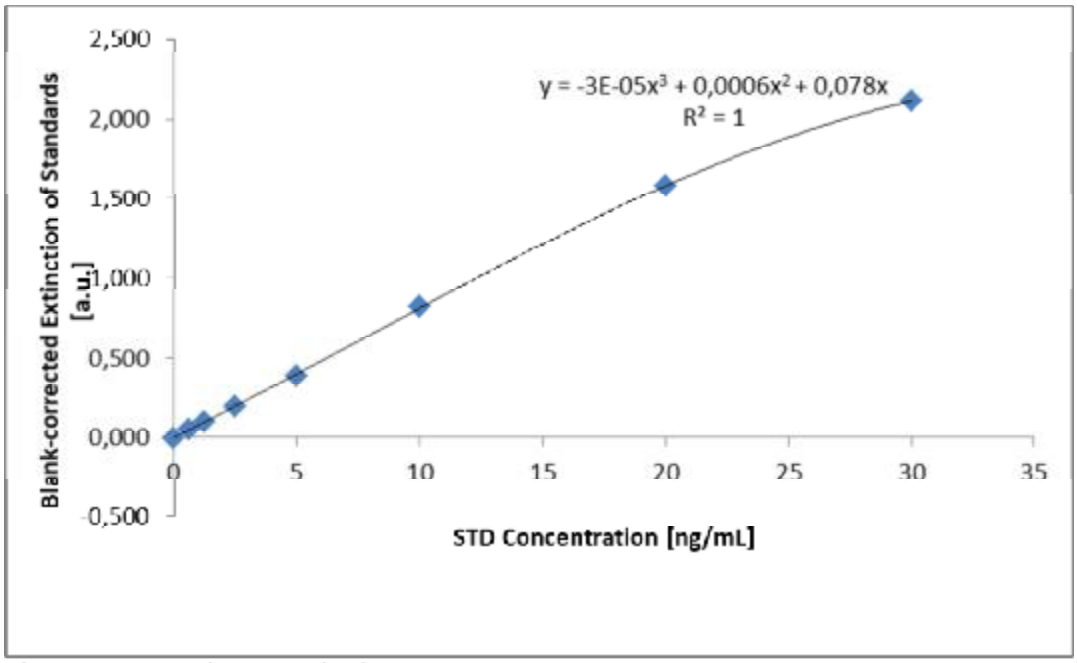


Figure 1: Exemplary Standard Curve

10.3 Exemplary evaluation of sample concentrations

Sample dilution: 1:5

Measured extinction of your sample: 0.419
Measured extinction of the Standard A: 0.00

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

$y = -3E\text{-}05x^3 + 0.0006x^2 + 0.078x$
$x = 5.17$

If the dilution factor (**5**) is taken into account the sLEP-R concentration of the undiluted sample is:

5.17 ng/mL x 5 = 25.85 ng/mL

11 EXPECTED VALUES

The expected values for sLEP-R were determined by ELISA Mediagnost R07 in samples of 39 healthy adult subjects (20 women, 19 men) and analyzed (Table 2).

Table 2: Expected values of sLEP-R in adults. The exemplary values were determined from samples from 39 healthy blood donors, shown is the gender mean, median, (n = number; MW = mean; SD = standard deviation; min = low; Max = maximum)

Gender	n	Mean [ng/ml]	SD [ng/ml]	VC [%]	Median [ng/ml]	Min. [ng/ml]	Max. [ng/ml]
Female	20	**24.2**	6.45	26.67	23.3	15.5	38.5
Male	19	**25.1**	7.22	28.73	24.1	14.1	36.7
Total	**39**	**24.6**	6.76	27.43	23.9	14.1	38.5

12 PERFORMANCE CHARACTERISTICS

12.1 Sensitivity

The analytical sensitivity of the test system is defined, as the concentration sLEP-R, that is distinguishable from the blank / background. Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. The analytical sensitivity of the Mediagnost sLEP-R ELISA is 0.0385 ng/mL on average.
The limit of quantification (LOQ, as Serial dilutions with 3 different sera with max. difference of ± 20% to expected value) is 0.56 ng/mL.

12.2 Specificity

Recombinant Leptin
Rec. sLEP-R was added at concentrations of 10 ng/mL to the Dilution Buffer VP, to this rec. Leptin in concentrations of 1 - 256 ng/mL was added. The sLEP-R content was measured: The measurement of rec. sLEP-R is not disturbed thorough rec. Leptin up to 256 ng/mL (values within +/-30%).

Table 3: Specificity The influence of the rec. Leptin was tested by adding to the rec. sLEP-R (10 ng/mL) in Dilution Buffer VP, rec. Leptin was added in concentrations between 1 and 256 ng/mL.

10 ng /mL sLEP-R = 12.38 ng/mL (determined)			
Rec. Leptin	**Target Value**	**Measured Value**	**Recovery**
[ng/ml]	**[ng/mL]**	**[ng/mL]**	**%**
1	12.38	10.0	80.9
2	12.38	11.3	91.2
4	12.38	12.2	98.4
8	12.38	12.3	98.5
16	12.38	12.3	99.2
32	12.38	11.3	91.0
64	12.38	11.7	94.5
128	12.38	11.2	90.7
256	12.38	8.8	71.5

12.3 Precision

Intra-Assay-Variance

Native human serum samples were measured (n=18) repeatedly at various positions of the microtiter plate. Intra-Assay variability was on average < 6%. Exemplary results are shown in Table 4.

Table 4: Intra-Assay Variance

	Sample 1	Sample 2	Sample 3
Mean [ng/ml]	**13.7**	**24.1**	**41.1**
SD	0.57	1.32	1.80
CV%	4.17	5.47	4.38
n	18	18	18

Inter-Assay Variance

Serum samples were measured in independent tests. The coefficient of variation was 8% on average. Exemplary results are shown in Table 5.

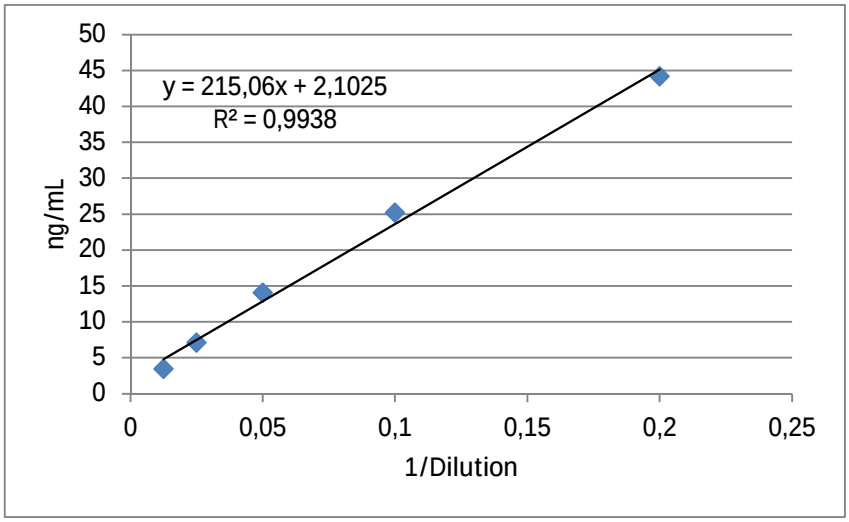
Table 5: Inter-Assay Varianz

	Sample 1	Sample 2	Sample 3	Sample 4
Mean [ng/mL]	**14.5**	**28.4**	**25.5**	**77.0**
SD	0.96	3.01	1.26	5.18
CV%	6.63	10.57	4.95	6.73
n	17	17	16	16

12.4 Linearity

The sLEP-R concentration of four human serum samples was determined at various dilutions. The results shown in Figure 2a and 2b prove that in the samples tested no influence of dilution can be detected. The linearity is given from the dilution 1:5 to 1:90.

2 a)



2 b)

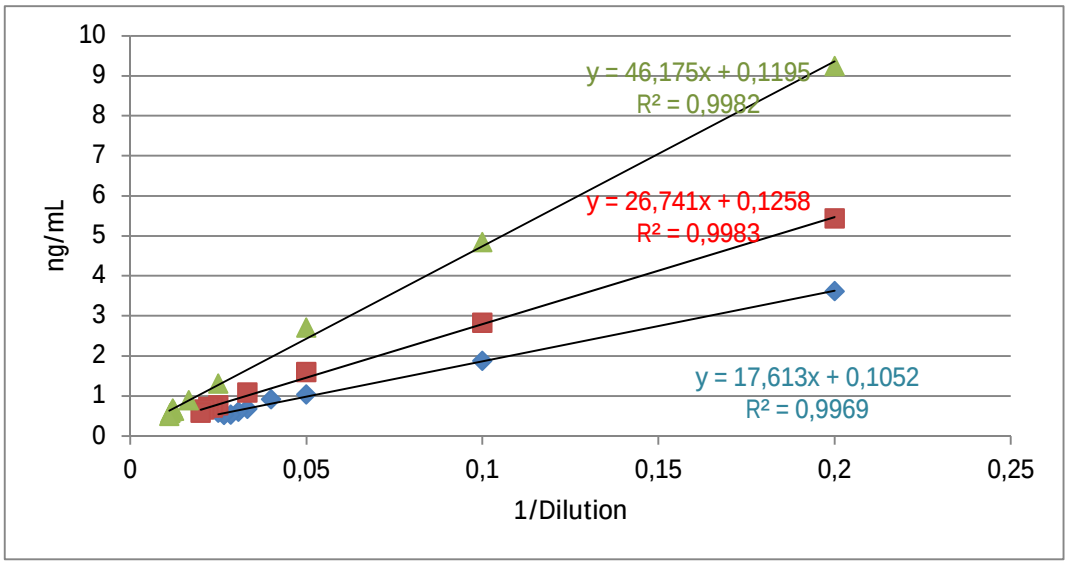


Figure 2a and 2b: Linearity, 4 serum samples were diluted in a range of 1:5 to 1:90 and the sLEP-R concentration in the respective dilutions was measured.

12.5 Interference

Triclycerides, Bilirubin and Hemoglobin

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

Table 6 Interference: The relative amount of measured sLEP-R as compared to native serum is [%] shown.

[%]	Triglycerides [100 mg/mL]	Bilirubin [100 µg/mL]	Hemoglobin [1 mg/mL]
Sample 1	95	107	103
Sample 2	96	110	96
Sample 3	103	86	116

12.6 Recovery and traceability

The recovery of recombinant sLEP-R in serum varied between 81 and 119% of the expected value

Table 7 Recovery of recombinant human sLEP-R in serum samples. Human serum samples were spiked with nominal 10 ng/mL recombinant sLEP-R, the concentration in R07 was determined, and recovery was calculated.

	Recovery in [%]			
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**
Sample 1:5 diluted	84	100	89	81
Sample 1:10 diluted	97	119	93	108

12.7 Calibration

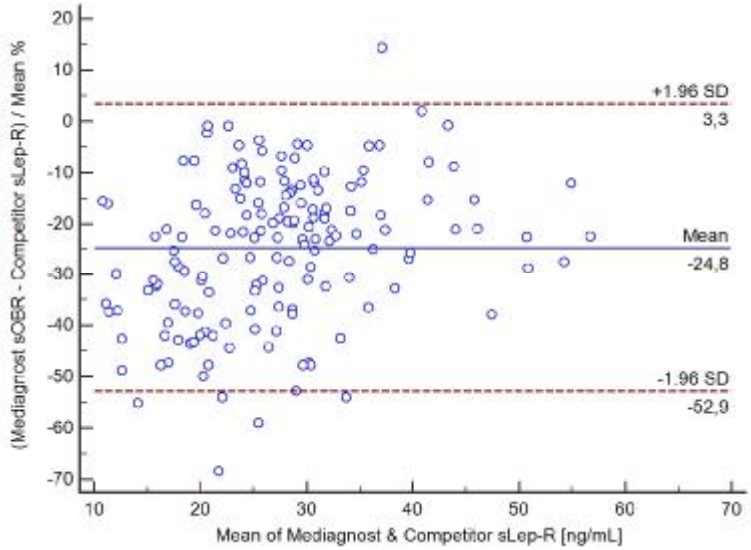
The calibration of the R07 was made against the recombinant Mediagnost sLEP-R (#P48357 AA20-839+HIS tag). The recovery relative to the nominal content of the recombinant Mediagnost standards averaged 96.4%.

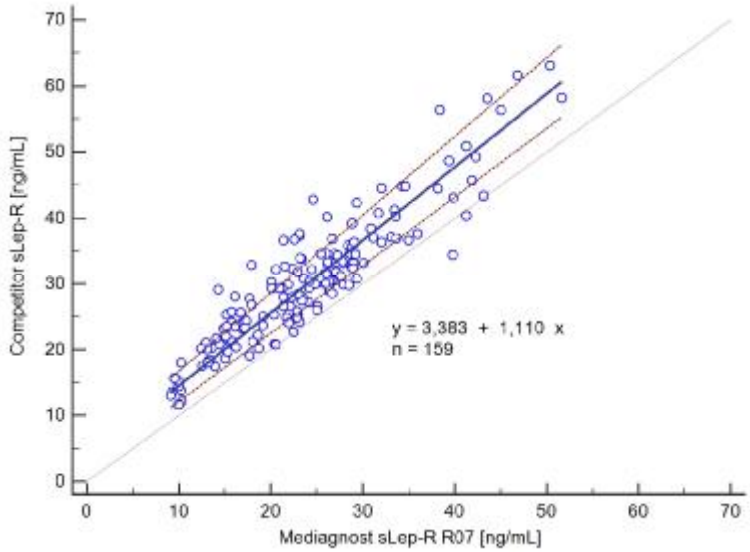
13 ASSAY COMPARISON

The measurement results of the Mediagnost sLEP-R R07 were compared with a commercially available competitor kit using a Passing-Bablock and Bland-Altman plot. For this purpose, the sLEP-R concentrations of 159 serum samples, clinical samples derived from children from age 6 years and adults and adult blood donors, were measured by both tests. The results are graphically shown in Figures 3a and 3b and prove that the mean deviation of the sLEP-R assay is 24.8% of the mean of both tests. The absolute height of the sLEP-R levels has no influence on the results.

The high degree of the linear relationship of both assay results is described by the Passing-Bablok curve equation of y = 3,382748 + 1.110451x.

3a) Bland-Altman-plot





3b) Passing-Bablok Regression

Figure 3a and 3b: Comparison Test R07 sLEP-R [ng/mL] values of 159 samples were compared with another commercially available kit. The results were analyzed by a) Bland-Altman plot and b) Passing-Bablok regression.

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16 SUMMARY OF THE ASSAY PROCEDURE R07

Preparation of reagents		**Reconstitution:**	**Dilution**
A-H	**Standards**	in **1 mL** Dilution Buffer **VP**	-
KS1	**Control Serum 1**	in **500 µL** Dilution Buffer **VP**	**1:5** with Dilution Buffer **VP**
KS2	**Control Serum 2**	in **500 µL** Dilution Buffer **VP**	**1:5** with Dilution Buffer **VP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**

Sample dilution: with Dilution Buffer **VP 1:5. Don´t use samples undiluted!**

Before assay procedure bring all reagents to room temperature **20-25°C**.

Assay Procedure in Double Determination:

Pipette	**Reagents**	**Position**
100 µL	Standard **A (0 ng/mL**)	A1/A2
100 µL	Standard **B (0.625 ng/mL**)	B1/B2
100 µL	Standard **C (1.25 ng/mL**)	C1/C2
100 µL	Standard **D (2.5 ng/mL**)	D1/D2
100 µL	Standard **E** (**5.0 ng/mL**)	E1/E2
100 µL	Standard **F (10 ng/mL**)	F1/F2
100 µL	Standard **G (20 ng/mL**)	G1/G2
100 µL	Standard **H (30 ng/mL**)	H1/H2
100 µL	Control Serum **KS 1** (1:5 diluted)	A3/A4
100 µL	Control Serum **KS 2** (1:5 diluted)	B3/B4
100 µL	Sample (1:5 diluted)	in the rest of the wells according the requirements
Cover the wells with the sealing tape.		
Sample Incubation: 2 hours at 20-25°C, 350 rpm		
5 x 300 µL	Aspirate the contents of the wells and wash **5 x** with **300 µL** each Washing Buffer **WP/ well.**	In each well
100 µL	Antibody Conjugate **AK**	In each well
Cover the wells with the sealing tape.		
Incubation: 2 hours at 20-25°C, 350 rpm		
5 x 300 µL	Aspirate the contents of the wells and wash **5 x** with **300 µL** each Washing Buffer **WP/ well.**	In each well
100 µL	Enzyme Conjugate **EK**	In each well
Cover the wells with the sealing tape.		
Incubation: 30 minutes at 20-25°C, 350 rpm		
5 x 300 µL	Aspirate the contents of the wells and wash **5 x** with **300 µL** each Washing Buffer **WP/ well.**	In each well
100 µL	Substrate Solution **S**	In each well
Incubation: 30 minutes in the Dark at 20-25°C		
100 µL	Stopping Solution **SL**	In each well
	Measure the absorbance within 30 min at **450 nm** with ≥ 590 nm as reference wavelength.	