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ALS-ELISA

Enzyme Immunoassay for Quantitative Determination of

human Acid Labile Subunit

English

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

2-8°C

96 wells

REF **E35**

mediagnost®
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	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
	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
	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
	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

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Instructions for use

ALS ELISA E35	96 Determinations
Regulatory status	For research use only, not for diagnostic procedures.
Principle of the test	Enzyme-linked Immunoassay
Duration (incubation period)	3 h
Antibodies	Specific, high-affinity polyclonal rabbit antibodies.
Buffer	Ready for use and 20fold concentrate
Standard	6 single standards: 0 – 200 ng/mL, native human ALS
Assay Range	0.53 – 30 000 ng/mL
Control	2 control sera, freeze-dried
Sample	human serum / plasma
Required sample volume	10 µL
Sample dilution	1:150
Analytical sensitivity	$\leq$ 0.53 ng/mL
Intra- / Interassay Variance	<10 %

1 INTENDED USE

This enzyme immunoassay kit is suited for measuring ALS in human serum or EDTA-/heparin-/citrate plasma for research use only. Not for use in diagnostic procedures.

2 INTRODUCTION

The Insulin-like Growth Factors (IGF) – I and II are bound to specific binding proteins in circulation (IGFBP). Until today seven different proteins have been identified IGFBP-1 to 7 [1, 2]. IGF bioavailablity, transport and storage is regulated or facilitated by these binding proteins which are expressed differentially according physiological and developmental requirements. The most abundant IGFBP in circulation is IGFBP-3. Together with IGFBP-5 it is able to form the so called ternary complex with IGF and the acid-labile subunit (ALS) [3-5]. In the circulation nearly all IGF is bound in this ternary complex and thus not able to cross the endothelial barrier. Only very small amounts of IGF or IGFBP-3 exist outside this complex [6, 7]. The acid-labile subunit is an important part of the IGF-storage mechanism in circulation. In ALS deficiency or in ALS knock-out mice the concentration of IGF and IGFBP-3 in the circulation is significantly decreased resulting in impaired growth [10].

The acid-labile Subunit, is a synthesized as propeptide of 605 amino acids. The signal peptide, necessary for ALS secretion (AA 1-27) cleaved off enduring the transport process (Swiss-Prot P35858 Version 82). The mature protein consists of 578 amino acids and contains about 20 leucin rich sequence repeats. Beside the leucin-rich repeats several potential N-linked glycosylation sides have been described. Miller BS et al. were able to demonstrate that incomplete glycolsylation of IGFs, ALS and IGFBP-3 results in a decreased serum concentration of these proteins. Mutations in or the complete knock out of the ALS gene result in IGF / IGFBP-3 deficiency [9,10].
The first ALS immunoassay was described by Baxter RC in 1990 [6]. By this in-house radioimmunoassay it was shown that ALS is present in high concentrations in serum (50µg/mL) of healthy humans. But not detectable in other body fluids like amniotic fluid, cerebrospinal fluid or seminal plasma – in spite of the fact that these body fluids contain high level IGFBP-3.

3 ASSAY PRINCIPLE

The Mediagnost ELISA for ALS **E35** is a so-called Sandwich-Assay. It utilizes two specific and high affinity antibodies for this protein. These antibodies were created by immunization of rabbits with specific peptides as previously described by Khosravi and Stadler [16, 17].

The ALS in the sample binds to the immobilized first antibody on the microtiter plate. The biotinylated second anti-ALS-Antibody binds also to the immobilized ALS. In the following step the Streptavidin-POD-Conjugate binds to the biotinylated antibody and in the closing substrate reaction the turn of the colour will be catalysed, quantitatively depending on the ALS-level of the samples.

Initially the test system was calibrated against an internal serum standard and measurement results were expressed as Mediagnost mU/mL. After successful production of eukaryotic recombinant ALS the calibration was transferred to mass units **(see Calibration / Traceability).**
Additionally recombinant material was used to quantify the ALS content of the calibrators in mass units. Thorough analysis revealed that **1 mU ALS is equivalent to 5 ng ALS** and all previous assay data describing the assay performance were accordingly transferred to ng/mL.

4 WARNINGS AND PRECAUTIONS

For research and professional use only.
The Mediagnost kit is suitable only for in vitro use and not 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. 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 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.

Human Serum
Following components contain human serum: **Control Sera KS1 and KS2, and Standards A-F**
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.

Reagents, AK, 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

Serum and Plasma

30 IU/mL Sodium Heparin, 3,8 g/L Sodium Citrate or 0,0068 mol/L EDTA did not interfere with ALS measurement.

5.2 Specimen collection

The blood sample for serum preparation should be gained according to standardized venipuncture procedure. Hemolytic reactions have to be avoided.

5.3 Required sample volume: 10 µL

5.4 Sample stability

In firmly closable sample vials

- Storage at 20-25°C: 3 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 freeze-thaw cycles showed no effect on the measured ALS concentration.

5.5 Interference

Hemoglobin, triglyceride and bilirubin in the sample do not interfere to a concentration of **1 µg/mL**, **100 mg/mL** and **200 µg/mL**, respectively. However, the use of hemolytic, lipemic or icteric samples should be validated by the user.

5.6 Sample dilution

- Dilution: **1:150** with Sample Buffer **PP**
- Pipette **1490 µL** Sample Buffer PP (red colored) in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), **add 10 µL Serum- or Plasma** (dilution 1:150) and mix each tube immediately. After mixing use 50 µL of this solution within 1 hour per determination in the assay (pipetting control = red coloring of the solution in the wells).
- Sample stability after dilution of the sample: maximum 1 hour at 20-25°C.
- In most determinations (e.g. Serum- or Plasma samples and no extreme values expected) the dilution of **1:150** with Sample Buffer **PP** is suitable, respectively the assay covers the range from **0.53 ng/mL - 30 µg/mL**. 1:50 is the minimal tested sample dilution.
- If required, the dilution with Sample Buffer PP could performed lower or higher.

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 rabbit-anti-ALS-antibody. Wells are separately breakable.	**(8x12) wells**
PP	**Sample Buffer PP,** ready for use, red colored, please use for the reconstitution of **Standards A-F** and **Controls KS1/KS2** and for the dilution of **Samples** and **Controls KS1/KS2.**	**1 x 125 mL**
A-F	**Standards**, lyophilized, (native human ALS), concentrations are given on vial labels and on quality certificate in ng/mL.	**6 x 1 mL**
KS1	**Control Serum 1,** lyophilised, (human serum), concentration is given on quality certificate in ng/mL.	**1 x 250 µL**
KS2	**Control Serum 2,** lyophilised, (human serum), concentration is given on quality certificate in ng/mL.	**1 x 250 µL**
VP	**Dilution Buffer,** ready for use, please use for the dilution of **Antibody Conjugate AK.**	**1 x 7 mL**
AK	**Antibody Conjugate, 50-fold concentrate**, contains the biotinylated anti-human rabbit ALS Antibody. Dilute before use **1:50 in Dilution Buffer VP** and use **50 µl** for each well in the assay. **Attention:** Please dilute **Antibody Conjugate AK** freshly according to daily requirements.	**1 x 140 µL**
EK	**Enzyme Conjugate EK, 12 mL**, ready-to-use, contains HRP (Horseradish-Peroxidase)-labeled Streptavidin.	**1 x 12 mL**
WP	**Washing Buffer,** 20-fold concentrated solution, dilute 1:20 in A. dest. or in deionized Water.	**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.**	**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 **WP (A. dest.),** 950 mL.
- Precision pipettes and multichannel pipettes with disposable plastic tips
- Polyethylene PE/Polypropylene PP tubes for dilution of samples
- Vortex-mixer
- Microtiter plate shaker (350 rpm)
- Microtiter plate washer (recommended)
- Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm

7 TECHNICAL NOTES

Storage Conditions

Store the kit at 2-8°C after receipt until its expiry date. 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** standards **A-F** and Control Sera **KS1 and KS2** must be stored at –20°C (max. 4 weeks). For further use, thaw quickly but gently (avoid temperature increase above room temperature and avoid excessive vortexing). Up to 3 of the freeze-thaw cycles did not influence the assay. The 1:20 diluted Washing Buffer **WP** is 4 weeks stable at 2-8°C

Preparation of reagents

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

Reconstitution

The Standards **A – F** and Control Sera **KS1** and **KS2** are reconstituted with the Sample Buffer **PP**. 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 Controls **KS1 and KS2** with the Sample Buffer **PP** in the same ratio **(1:150)** as the sample.

The required volume of **Antibody Conjugate AK** is prepared by **1:50** dilution of the provided 50-fold concentrate with **Dilution Buffer VP.** Please dilute Antibody Conjugate freshly according to daily requirements.

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-F**, Controls **KS1, KS2** and the samples should be pipette as fast as possible (e.g. <15 minutes). To avoid distortions due to differences in incubation times, 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-F**, Controls **KS1**, **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 H_2O_2-Tetramethylbencidine, is photosensitive–store and incubation in the dark.

Shaking

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

Washing

Proper washing is of basic **importance** for a secure, reliable and precise performance of the test. Incomplete washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.

All washing must be performed with the provided Washing Buffer **WP** diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least.

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

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

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

8 ASSAY PROCEDURE

Preparation of reagents		Reconstitution:	Dilution
AK	**Antibody-cojugate**	-	Dilute before use with **1:50** Dilution Buffer **VP**
A-F	**Standards**	in **1 mL** Sample Buffer **PP**	-
KS1 and KS2	**Control Sera**	In **250 µL** Sample Buffer **PP**	**1:150** with Sample Buffer **PP**
WP	**Washing Buffer**	-	**1:20** with **Aqua dest.**

Sample and Control Sera KS1 &KS2 Dilution: 1:150 in Sample Buffer PP (red colored; e.g. **10 µL** in **1490 µL PP**). **Mix directly and use within max. 60 min.**
Use **50 µL per determination** (pipetting control= red coloration)

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

Assay Procedure in Double Determination:

Pipette	Reagents	Well Position
50 µL	**1:50 diluted Antibody Conjugate**	**Pipette in <u>all</u> required number of wells**
50 µL	Standard **A** (**0 ng/mL**)	A1/A2
50 µL	Standard **B** (**7.5 ng/mL**)	B1/B2
50 µL	Standard **C** (**31.25 ng/mL**)	C1/C2
50 µL	Standard **D** (**62.5 ng/mL**)	D1/D2
50 µL	Standard **E** (**125 ng/mL**)	E1/E2
50 µL	Standard **F** (**200 ng/mL**)	F1/F2
50 µL	Control Serum **KS1** (1:150 diluted)	G1/G2
50 µL	Control Serum **KS2** (1:150 diluted)	H1/H2
50 µL	Sample (1:150 diluted)	in the rest of the wells according the requirements
Cover the wells with the sealing tape.		
Sample Incubation: **2 h at 20-25°C**, **350 rpm**		
5 x 300 µL	Aspirate the contents of the wells and **wash** 5 x with 300 µL each Washing Buffer **WP/ well**	In each well
100 µL	**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

Good laboratory practice requires that controls are included in each assay. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The test results are only valid if the test has been performed following the instructions. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable federal, state or local standards/laws. All standards and 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. Each laboratory should use known samples as further controls.

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 F** should be above **1.00.**
Samples, which yield higher absorbance values than **Standard F**, should be re-tested with a higher dilution.

10 EVALUATION OF RESULTS

10.1 Establishing of the standard curve

Standard	A	B	C	D	E	F
ng/mL	0	7.5	31.25	62.5	125	200
mU/mL	0	1.5	6.25	12.5	25	40

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 absorbance 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 ALS concentration in ng/mL of the samples and controls **KS1** and **KS2** 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.

	A	B	C	D	E	F
ng/mL	0	7.5	31,25	62,5	125	200
$OD_{(450\text{-}620\ nm)}$	0.049	0.282	1.248	1.929	2.54	2.934

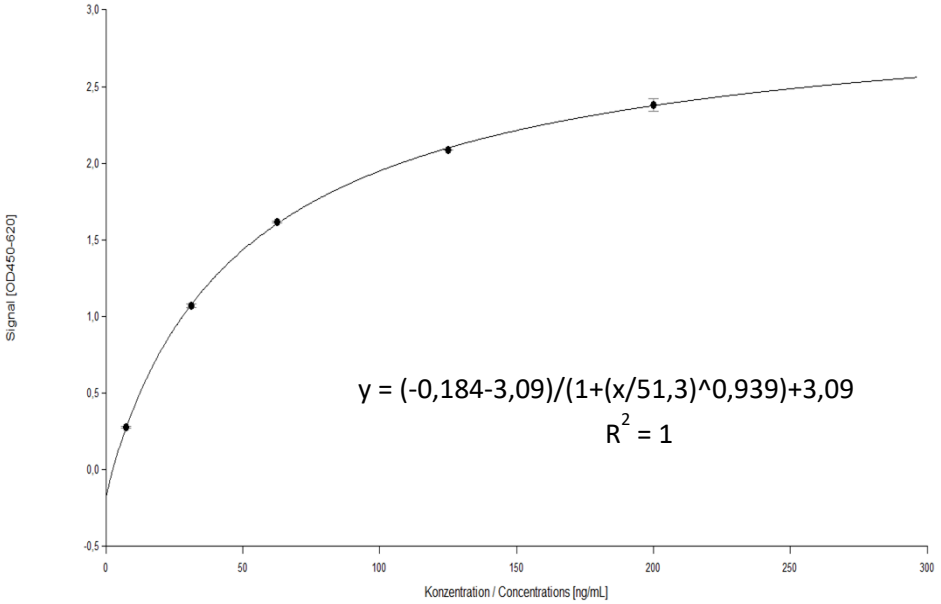


Figure 1 Examplary standard curve

The exemplary shown standard curve in Figure 1 **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 ALS concentrations

Sample dilution: 1:150

Measured extinction of your sample 1.5
Measured extinction of the Standard A 0.049

Your measurement programm will calculate the ALS concentration of the diluted sample automatically by using the difference of extinction values 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 ALS concentration in the sample:

$$1.451 = (-0.184\text{-}3.09)/(1+(x/51.3)^{0.939})+3.09$$

$$51.201 = x$$

If the dilution factor (1:150) is taken into account the ALS concentration of the undiluted sample is

$$51.201 \times 150 = 7680\ ng/mL$$

10.4 Limitation of procedure

The Mediagnost sensitive human **ALS ELISA**, **E35** is based on antibodies. Generally, this technique could be sensible to heterophilic antibodies or rheumatic factors in the sample. Their influence is reduced by assay design, but cannot be excluded completely.

11 EXAMPLARY VALUES

Serum samples of healthy blood donors were used to assess concentration in healthy adult humans. Significant differences between sexes were not detected and an age dependency was not evaluated.

Table 1 Examplary values for adults in serum.

	male [ng/mL]	female [ng/mL]
Mean	**7095**	**8413**
SD	**1252**	**1956**
Median	7162	8236
Min	4525	5332
Max	10031	11981
n	39	35

12 PERFORMANCE CHARACTERISTICS

12.1 Calibration - Traceability

No international standard or reference preparation of ALS is available. Initially, the Mediagnost ALS ELISA was calibrated against a human serum standard. In a second step the test system was recalibrated with eukaryotic, recombinant ALS. The recombinant ALS was measured in three different Mediagnost E35 lots. A comparison of the measured results is shown in Figure 2. The analysis revealed a factor of 5 to transfer Mediagnost Units in mass units (ng/mL).
According to the function y = 4,997x, the factor of 5 is used in the conversion of Mediagnost Units (mU/mL) in mass units (ng/mL).
1 Mediagnost Unit ALS ≈ 5 ng rec. ALS

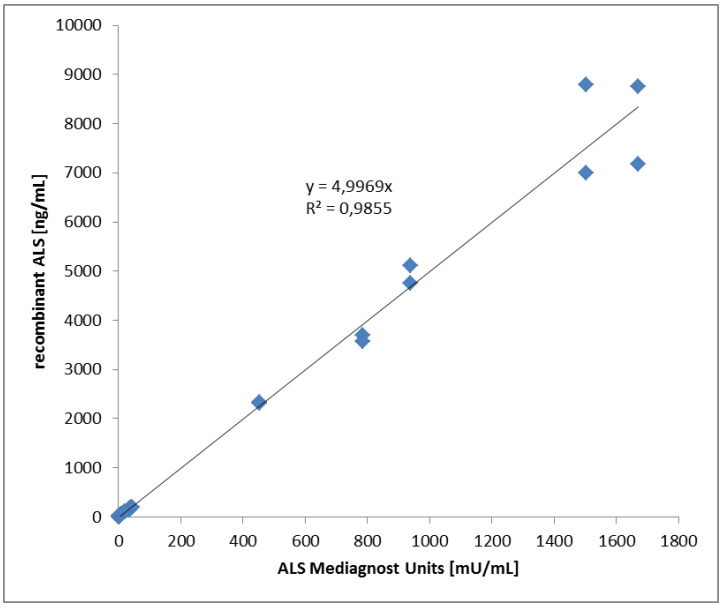
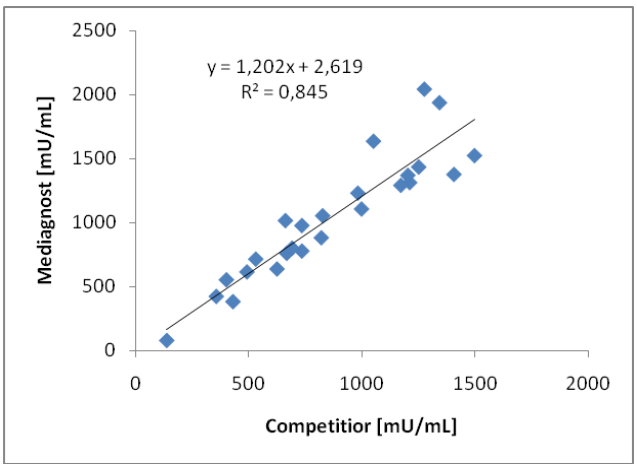


Figure 2 Assay Calibration, relation of Mediagnost Units and mass units.

A previously conducted comparative analysis of serum samples demonstrates that the Mediagnost E35 measures comparable results referring to an in-house assay used by an academic group (see Figure 3).



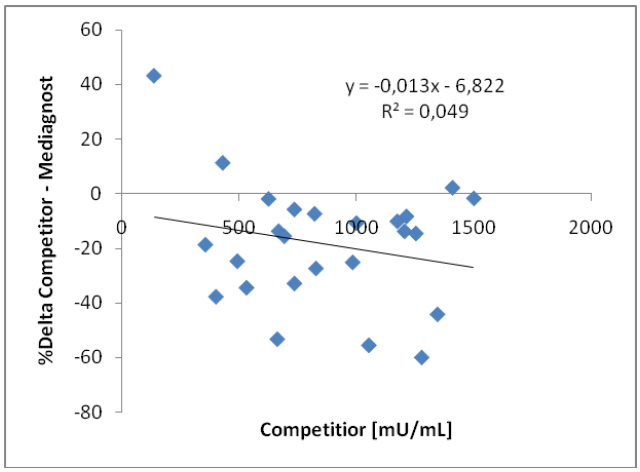


Figure 3 Comparative analysis of a competitive immunoassay with the Mediagnost E35 (serum samples: n=25).

12.2 Sensitivity

Sensitivity was assessed by measuring the blank and calculating the theoretical concentration of the blank + 2SD. In three measurements a range of 0.15 – 1.15 ng/mL with a mean sensitivity of 0.53 ng/mL was detected.

12.3 Precision Data

Intra-Assay Variance

Two samples have been measured 22 times in the same assay. The results are shown in Table 2. The measured coefficient of variation (CV) is 6.7% on average.

Table 2 Intra-Assay Variation

	Number of determinations	Mean value [ng/mL]	Standard deviation [ng/mL]	VC [%]
Sample 1	22	4556	298	6,55
Sample 2	22	6694	458	6,84

Inter-Assay Variance

Serum samples were measured in independent assays. On average the coefficient of variation was 8.96% (SD 6.11). Exemplary results are shown in table 3.

Table 3 Inter-Assay Variation

	Number of single determinations	Mean value [ng/mL]	Standard deviation [ng/mL]	VC [%]
Sample 1	39	4980	485	10
Sample 2	45	5530	525	9
Sample 4	12	3225	230	7

12.4 Linearity

Linearity was tested by dilution of three native serum samples with high ALS content. The optical density of each dilution was measured and the results are shown in Figure 4. Serial dilution of three samples within a range of 1:50 – 1:500 revealed a good linearity measured by linear regression analysis (R^2>0.95).

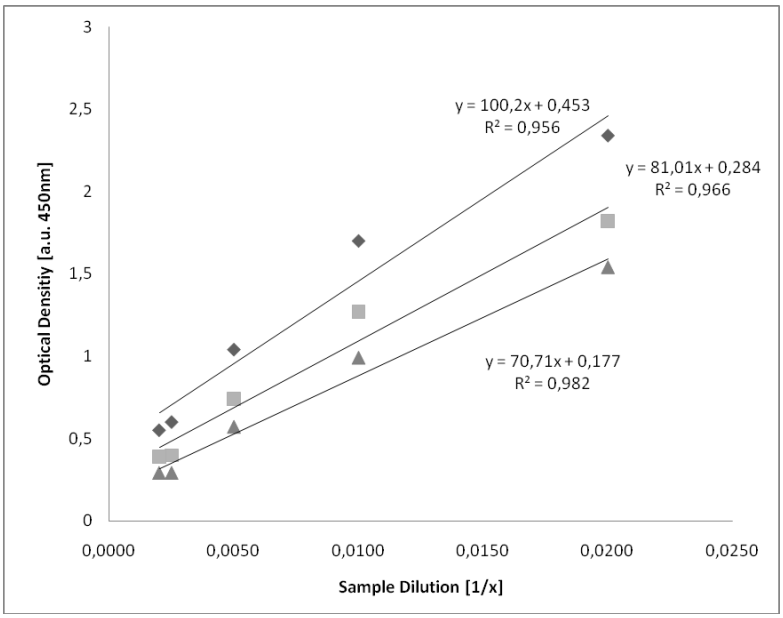


Figure 4 Linearity, measured signal intensity [OD_{450}] of differentially diluted samples. The recommended dilution is 1:150 (0.007).

12.5 Interference

Interference of bilirubin and triglycerides was tested by adding different amounts of these substances to human serum containing ALS. For comparison the same amount of buffer without any substance was also added to the serum. Table 4 demonstrates that none of the tested substances exert any influence on the measurement of ALS in human serum.

Table 4 Interference

Triglyceride [mg/mL]	**0**	**12,5**	**25**	**50**	**100**
ALS [ng/mL]	5809	5403	5780	5383	5813
Bilirubin [µg/mL]	**0**	**25**	**50**	**100**	**200**
ALS [ng/mL]	5809	5283	5431	5771	5439
Hemoglobin [µg/mL]	**0**	**0.125**	**0.25**	**0.5**	**1**
ALS [ng/mL]	5809	5667	5315	6015	6100

12.6 Species Cross-Reactivity

Several commercially available animal sera have been diluted 1:10 and the diluted specimens were used as samples in this assay. Only light signals were detected in serum samples of chicken, cattle, dog, rat, donkey, mouse, goat, sheep, guinea pig, fetal calve serum. On average signal intensity was about 0.1 (corresponding Standard A value: 0.04).

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14 Internationale Assay Description

AK	**Ab**		**1:50 DILU BUF VP**
A-F	**STD**	**Rec in 1 mL BUF PP**	**-**
KS1	**Control**	**Rec in 250 µL BUF PP**	**1:150 DILU BUF PP**
KS2	**Control**	**Rec in 250 µL BUF PP**	**1:150 DILU BUF PP**
WP	**WASHBUF 20x**	**-**	**1:20 DILU A. dest.**
-	**SPE**		**1:150 DILU BUF PP**
-	**°C 20-25 °C**		

50 µL	**Ab AK 1:50 DILU BUF VP**	**A1 -End**
50 µL	**STD A (0 ng/mL)**	A1/A2
50 µL	**STD B (7.5 ng/mL)**	B1/B2
50 µL	**STD C (31.25 ng/mL)**	C1/C2
50 µL	**STD D (62.5 ng/mL)**	D1/D2
50 µL	**STD E (125 ng/mL)**	E1/E2
50 µL	**STD F (200 ng/mL)**	F1/F2
50 µL	**CONTROL KS1 1:150 DILU BUF PP**	G1/G2
50 µL	**CONTROL KS2 1:150 DILU BUF PP**	H1/H2
50 µL	**SPE 1:150 DILU BUF PP**	
	TAPE	
	2 h °C 20-25 ↔ 350 rpm	
5x 300 µL	**5x WASHBUF WP**	
100 µL	**Conj EK**	
	TAPE	
	0.5 h °C 20-25 ↔ 350 rpm	
5x 300 µL	**5x WASHBUF WP**	
100 µL	**SUBST TMB S**	
	0.5 h °C 20-25	
	H_2SO_4 SL	
	MEASURE	