Vaspin ELISA

Enzyme Immunoassay for quantitative Determination of

human VASPIN

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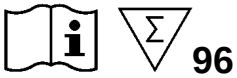
Not for use in diagnostic procedures.



REF **E106**

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Symbols/ Symbole /Symboles/ Simboli/ Símbolos/ Símbolos/ Symbolen/ Symboler/ Symboler/ Symbole/ Szimbólumok/ Symboly/ Symboly/ Символи/ Sümbolid/ Σύμβολα/ Simboluri/ Simboli/ Symbolit

according to DIN EN 980 and EDMA recommendations Standard News 6 2001

Expiry date/ Verfallsdatum/ Date de péremption/ Data di scadenza/ Fecha de caducidad/ Data de validade/ Uiterste gebruiksdatum/ Udløbsdato/ Bäst före-datum/ Termin ważności/ Lejárati idő/ Čas exspirácie/ Doba exspirace/ Срок на годност/ Aegumiskuupäev/ Ημερομηνία λήξης/ Data de expirare/ Rok uporabe/ Viimeinen käyttöpäivä

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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

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

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/ suojaa 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

Shaking/ Schütteln/ Mélanger/ Agitare/ Agitar/ Agitação/ Schudden/ Ryster/ Skaka/ Wstrząsanie/ Rázás/ Pretrepať/ Protřepat/ Разклащане/ Raputada/ Ανακινήστε/ Vibrare/ Stresite/ Sekoita

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

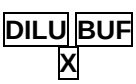
Reconstitute in/ Rekonstituieren in/ Reconstituer dans/ Ricostituire nel/ Reconstituir en/ Reconstituir em/ reconstitueren in/ Rekonstituér i/ rekonstituera/ Rekonstytuować w/ Helyreállítás/ Znovu pripraviť za/ Znovu připravit za/ Разтваряне в/ Moodustada uuesti / Ανασυστήστε σε/ Reconstituire în/ Predelava v/ rekonstituoi

Sample/ Probe /Echantillon/ campione/ Muestra/ Amostra/ monster/ Prøve/ prov/ Próbka/ Minta/ Vzorka/ Vzorek/ Проба/ Proov/ Δείγμα/ Probă/ Vzorec/ Näyte

AK Antibody and Enzyme Conjugate/ Antikörper und Enzym Konjugat/ anticorps conjuguée et conjugué enzymatique/ Coniugato di anticorpo ed enzima/ Conjugado de anticuerpos y enzimas/ Conjugado Anticorpo-Enzima/ antilichaam-en enzymconjugaat/ Antistoffer og enzym-konjugat/ antikropps- och enzymkonjugat (antikropp och enzym, konjugat)/

EK Koniugat antyciał i enzymów/ Antitest és enzim páros/ Protilátkový a enzymatický konjugát/ Protilátkový a enzymatický konjugát/ Антитяло и ензим конюгат/ Antikehad ja ensüümi konjugaat/ Σύμπλοκο αντισώματος-ενζύμου/ Compuşi din anticorpi şi enzime/ Antitelesa in konjugat encima/ Vasta-aine ja entsymi konjugaatti

VP Buffer / puffer/ Tampon / Tampone / Tampón /Tampão / buffer/ buffer/ buffert/ Bufor / puffer/ pufer/ pufr/ буфер/ puhver/ ρυθμιστικό διάλυμα / Tampon /pufer/ puskuriliuos



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

A-E 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/ Kalibraattori X

KS1
KS2 Control Serum / Kontrollserum/ Contôle sérique/ Siero di controllo/ Suero de control/ Soro de Controlo/ controleserum/ Kontrolserum/ Kontrollserum/ Serum kontrolne/ Ellenőrző szérum/ Kontrolné sérum/ Kontrolní sérum/

		Контролен серум/ Kontrollseerum/ Ορός ελέγχου/Ser de control/ Kontrolni serum/ Kontrolli seerumi
WASHBUF 20x	WP	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	S	Substrate/ Substrat/ Substrat/ Substrato/ Substrato/ Substrato/ substraat/ Substrat/ Substrat/ Substrat/ Szubsztrátum/ Substrát/ Substrát/ Субстрат Substraat/ Υπόστρωμα/ Substrat/ Substrat/ Substraattiliuos
H_2SO_4	SL	Stop Solution/ Stopp Lösung/ 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 ≥590nm)/Ausmessung innerhalb von 30 min bei 450 nm (Referenzfilter ≥ 590 nm)./ Mesure lábsorbance en léspace de 30 min à450 nm avec ≥590nm 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 ≥590nm)/ Binnen 30 minuten bij 450 nm meten (referentiefilter ≥ 590 nm)./ Mål plade i løbet af 30 min ved nm (referencefilter ≥590nm)/ 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/Měřit 30 minut při 450 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

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

PACKAGE INSERT ENGLISH

Mediagnost Vaspin ELISA E106

- is suited for Vaspin determination in **serum** samples
- is extremely **sensitive** (Limit of Blank **4 pg/ml**)
- is **fast**: incubation time a total of 3.5 hours
- Single Standards with **10, 75, 200, 500,1000 pg/ml** human Vaspin are provided in the kit
- 2 Control Sera are provided for quality control purposes according GLP
- is calibrated with **recombinant Vaspin**
- Microtiter plates are separately break apart, tests can be adapted to individual requirements

INTENDED USE

Measurement of human Vaspin human serum. For Research Use Only. Not for Diagnostic Procedures.

INTRODUCTION

The ELISA for quantitative measurement of Vaspin is based on polyclonal rabbit antisera raised by genetic immunization of the rabbits (cDNA Vaspin SC306941).

Vaspin also known as SerpinA12 is a serine protease inhibitor and consists of 395 amino acids forming 3 β-sheets and 9 α-helices. Molecular weight of Vaspin is about 45.2 kDa. It does not form multimeric aggregates or intra-molecular disulfide bridges and no binding proteins in human serum are known. The Vaspin gene is not only expressed by subcutaneous and visceral adipose but also by liver tissue, in the pancreas[1] and in the human epidermis (granular keratinocytes / GK cells)[2].

“Serum Vaspin levels were **highest in the early morning** before breakfast and fell to trough levels within 2 h after breakfast. Serum Vaspin levels also showed a preprandial rise and postprandial fall at lunch and dinner, although at lesser degrees than at breakfast. Intermeal Vaspin concentrations reached a **nadir in the mid-afternoon** and showed a nocturnal rise, with peak nighttime Vaspin levels being approximately 250% of nadir levels. Unscheduled food ingestion after a prolonged fast significantly reduced serum Vaspin levels, suggesting that energy intake itself has a suppressive effect on serum Vaspin levels. The diurnal pattern of serum Vaspin concentrations was exactly reciprocal to that of insulin and of glucose” [3].

A sexual dimorphism has been detected with higher Vaspin levels in girls increasing with age and pubertal stage[1,4]. A preliminary investigation (n = 81) of Vaspin levels in healthy adult blood donors revealed higher Vaspin levels in women decreasing with increasing age. In this context it is important to reflect, that oral contraceptives significantly increase serum Vaspin concentration[4].

Serum Vaspin concentration is independent of BMI but negatively associated with insulin sensitivity and obesity, thus “Vaspin was increased with worsening insulin resistance” [1]. If glucose metabolism / insulin sensitivity is improved by therapeutic intervention e.g. rosiglitazone, plasma Vaspin concentration decreases significantly[5]. Interestingly lifestyle intervention results in increasing adiponectin concentrations as well as in improved insulin sensitivity but after a 10 months intervention Vaspin concentration remain unchanged[6]. Thus,

the mechanism regulating the Vaspin concentration in circulation is still unclear. Vaspin concentration might be even more influenced by glucose uptake than by body fat at least in pre-pubertal children[7].
In insulin resistance, diabetes as well as atherosclerosis inflammatory processes are involved and Vaspin might be a link between the endocrine and the immune system. To elucidate the role of Vaspin in inflammation its influence on TNF-α-stimulated production of reactive oxygen species was investigated in smooth muscle cells. Vaspin significantly decreased the TNF-α-induced monocyte adhesion to SMCs as well as TNF-α induced intracellular signal cascade [8].

The diagnostic value of Vaspin remains unclear, conflicting results question its value as biomarker for visceral or total adipose tissue. As well as regarding insulin resistance while in children Vaspin might correlate with insulin sensitivity[1] but in adults no correlation was found[4].

The Mediagnost Vaspin ELISA is a tool for the further investigation and validation of Vaspin as a biomarker for the visceral adipose tissue, insulin sensitivity and glucose tolerance.

MATERIALS PROVIDED

1)	MTP	**Microtiter plate**, ready for use, with 96 wells, dived up in 12 stripes à 8 wells (separately break apart), coated with anti-human Vaspin antibody.
2)	STD	**Standards A-E, lyophilised**, contain recombinant **Vaspin. Standard values are between 0.010 – 1 ng/ml** (10, 75, 200, 500, and 1000 pg/ml) **Vaspin** and have to be reconstituted with **750 µl (each) Dilution Buffer VP.** Use 100 µl pro well in the assay.
3)	BUF	**Dilution Buffer VP, 120 ml,** ready for use, after shaking. Please use this for the **reconstitution** of **Standards** and **Control Sera** and for the **dilution** of **Control Sera** and **Samples**.
4)	Control	**Control Sera KS1 and KS2**, **250 µl,** lyophilised, contain human serum and should be **reconstituted in each 250 µl Dilution Buffer VP**. The Vaspin target values and the respective ranges are given on the vial labels. The dilution should be according to the dilution of the respected samples. Use **100 µl** pro well in the assay.
5)	Ab	**Antibody Conjugate AK, 12 ml**, ready for use, contains the biotinylated anti-Vaspin antibody. Use 100 µl for each well in the assay.
6)	CONJ	**Enzyme Conjugate EK, 12 ml**, ready for use, contains horseradisch-peroxidase conjugate to streptavidin, Use 100 µl for each well in the assay.
7)	WASHBUF 20x	**Washing Buffer (WP), 50 ml, 20-fold concentrated solution**. **Washing Buffer (WP)** has to be diluted 1:20 with distilled or demineralised water before use (e.g. add the complete contents of the flask (50 ml) into a graduated flask and fill up with A. dest. to 1000 ml). Attention: After dilution the Washing Buffer is only 4 weeks stable, dilute only according to requirements.
8)	SUBST	**Substrate (S), 12 ml**, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2 Tetramethylbencidine.
9)	H_2SO_4	**Stopping Solution (SL), 12 ml,** ready for use, 0.2 M sulphuric acid, Caution acid!
10)		Sealing tape for covering of the microtiter plate, 3 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

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

WARNINGS AND PRECAUTIONS

For research and professional use only.

Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.

Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.

Use separate pipette tips for each sample, control and reagent to avoid cross contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
Do not use expired reagents. Do not use obvious damaged or microbial contaminated or spilled material.

Disposal of containers and unused contents should be done in accordance with federal and local regulatory requirements.

General first aid procedures:
Skin contact: Wash affected area thoroughly with water. Discard 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: If swallowed, wash out mouth thoroughly with water. Immediately see a physician.

Caution: This kit contains material of human and/or animal origin. The components and samples should be treated as potentially infectious.

Do not eat, drink or smoke in these areas. Never pipette the materials with the mouth. Avoid direct contact with these materials by wearing laboratory coats and disposable gloves. Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

Human Serum
Contained in following components: **Control Serum KS1 and KS2.**
The sources of human sera were tested and found non-reactive for Hepatitis-B surface antigen (HBsAg), Hepatitis C virus (HCV), and Human Immunodeficiency Virus 1 and 2 (HIV) antibodies. No known test methods can offer total assurance of the absence of infectious agents; therefore all components and patient's specimens should be treated as potentially infectious.

The reagents **A-E, AK, EK, VP, WP** contain **< 0.01% 2-Methyl-4-isothiazolin-3-one** and / or **< 0.01% 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-Isothiazol-3-one** as preservative:

Do not ingest. Wear suitable protective clothing and gloves. Avoid contact with skin, eyes and clothing. In case of contact with eyes or skin, wash immediately with plenty of water. In case of ingestion or eye contact, seek medical advice.

Stop solution contains 0.2 M Sulfuric Acid (H_2SO_4)

R36/38	Irritating to eyes and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves.

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine. Store and incubate in the dark.

R20/21/R22	Harmful by inhalation, in contact with skin and if swallowed
R36/37/38	Irritating to eyes, respiratory system and skin
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S28.1	After contact with skin, wash immediately with plenty of water
S36/37	Wear suitable protective clothing and gloves

METHOD

The enzyme immunoassay for Vaspin E106 is a so-called Sandwich-Assay. It utilizes specific and high affinity polyclonal antibodies for this protein. The Vaspin in the samples binds to the immobilized first antibody on the microtiter plate. In the following step, the biotinylated antibody binds in turn to Vaspin. After washing, Streptavidin-Peroxidase-Enzyme conjugate will be added,

which will bind highly specific to the biotin and will catalyse the enzymatic reaction, which turns the colour of the substrate, quantitatively depending on the Vaspin level of the samples.

SPECIMEN

Serum samples can be used in this assay.

Storage of the samples

Storage at –20°C in tightly closable plastic tubes is recommended.

Sample Preparation

Samples have to be diluted in Dilution Buffer (VP). For most of the determinations (serum samples, and no extreme values are expected) a dilution **of 1:4 with Dilution Buffer VP** should be suitable. According to expected Vaspin levels the dilution with VP can be higher or lower. The excellent linearity of this test system allows sample dilution of 1:2 to 1:32.

Vaspin concentrations may be completely different in body fluids of human origin other than serum or cell culture supernatants.

Suggestion for dilution protocol:
Pipette **210 µl Dilution Buffer VP** in PE-/PP-Tubes (application of a multi-stepper is recommended in larger series), add **70 µl Serum- or Plasma** (dilution1:4). After mixing use 100 µl per determination of this dilution in the assay.

TECHNICAL RECOMMENDATIONS

Reagents with different lot numbers cannot be mixed. All reagents and micro titerplate are stable until the indicated expiry, if stored unopened and protected from sunlight at 2 – 8°C.

The shelf life of the components after opening is 4 weeks, if used appropriately.

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.

Incubation at room temperature means: 20-25°C

The incubation steps should be performed at mean rotation frequency of a particularly suitable microtitre plate shaker. We are recommending 350 rpm. Due to certain technical differences deviations may occur, in case the rotation frequency must become 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.

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 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 dynamically 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.

Standards and Controls

For the reconstitution of the lyophilised **Standards A - E Dilution Buffer VP** has to be used.

The lyophilised **Control Sera KS1 and KS2** must be **reconstituted** with the **Dilution Buffer VP**. The dilution should be according to the dilution of the respected samples.

It is recommended to keep reconstituted reagents at room temperature for 15 minutes and then to mix them thoroughly but gently (no foam!) with a Vortex mixer.

The reconstituted standards and controls can be stored for 1 month at –20°C. Repeated freeze/thaw cycles have to be avoided.

Washing Buffer

The required volume of Washing Buffer is prepared by 1:20 dilution of the provided 20-fold concentrate with deionised water. The diluted Washing Buffer is stable for 4 weeks at 2-8°C. It has to be at room temperature for usage!

Microtiter plate

Store the once unused microtiter strips and wells together with the desiccant in the tightly closed clip lock bag at 2-8°C use in the frame provided. After opening the microtiter plate is stable for 4 weeks.

Substrate Solution

The Substrate Solution (S), stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

ASSAY PROCEDURE

When performing the assay, the Standards **A-E,** Control Sera **KS1& KS2** and the samples should be pipetted as fast as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times, 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. **Stop Solution SL** should be added to the plate in the same order as the Substrate Solution **S**
All determinations (Standards, Control Sera and samples) should be assayed in duplicate.

For optimal results, accurate pipetting and adherence to the protocol are recommended.

1) Pipette in positions A1/2 **100 µl Dilution Buffer VP (blank).**
2) Pipette in positions B1/2 **100 µl of the Standard A (10 pg/ml),**

   pipette in positions C1/2 **100 µl of the Standard B (75 pg/ml)**,

   pipette in positions D1/2 **100 µl of the Standard C (200 pg/ml)**,

   pipette in positions E1/2 **100 µl of the Standard D (500 pg/ml)**,

   pipette in positions F1/2 **100 µl of the Standard E (1000 pg/ml)**.

   To control the correct accomplishment of the assay **100 µl** of the 1:4 (or in respective dilution ratio of the samples) in Dilution Buffer VP diluted **Control Sera KS1/KS2** can be pipetted in positions G1/2 and H1/2.

   Pipette **100 µl** each of the diluted samples (e.g. dilute 1:4 with **Dilution Buffer VP**) in the rest of wells, according to your requirements.
3) Cover the wells with sealing tape and incubate the plate for **1 hour** at **room temperature** (shake at 350 rpm).
4) After incubation aspirate the contents of the wells and wash the wells 5 times **300 µl Washing Buffer WP** / well.
5) Following the last washing step pipette **100 µl** of the **Antibody Conjugate AK** in each well.
6) Cover the wells with sealing tape and incubate the plate for **1 hour** at **room temperature** (shake at 350 rpm).
7) After incubation aspirate the contents of the wells and wash the wells 5 times **300 µl Washing Buffer WP** / well.
8) Following the last washing step pipette **100 µl** of the **Enzyme Conjugate EK** in each well.
9) Cover the wells with sealing tape and incubate the plate for **1 hour** at **room temperature** (shake 350 rpm).
10) After incubation wash the wells 5 times with Washing Buffer **WP** as described in step 4.
11) Pipette **100 µl** of the **Substrate Solution S** in each well.
12) Incubate the microtiter plate for **30 minutes in the dark** at **room temperature**.
13) Stop the reaction by adding **100 µl Stopping Solution SL** to all wells.
14) Measure the absorbance within **30 minutes at 450 nm (Reference filter ≥ 590 nm)**

ESTABLISHING THE STANDARD CURVE

For the evaluation of the assay it is preconditioned that the absorbance values of the blank should be below 0.25, these of standard E should exceed 0.8.

Samples, which yield higher absorbance values than Standard E are beyond the standard curve, for reliable determinations these samples should be tested anew with a higher dilution.

The standards provided contain the following concentrations of Vaspin:

Standard	**A**	**B**	**C**	**D**	**E**
ng/ml	0.010	0.075	0.20	0.5	1.0
pg/ml	10	75	200	500	1000

1) Calculate the mean absorbance (MA) value for the blank from the duplicated determination (well A1/A2).
2) Subtract the mean absorbance (MA) 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 e.g. **a linear regression,** 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 **Vaspin concentration** of the diluted sample or the diluted control sera in pg/ml (or ng/ml according the chosen unit for the standards) is calculated in this way, the Vaspin concentrations of the **undiluted samples** and of control sera are calculated **by multiplication with the respective dilution factor**.

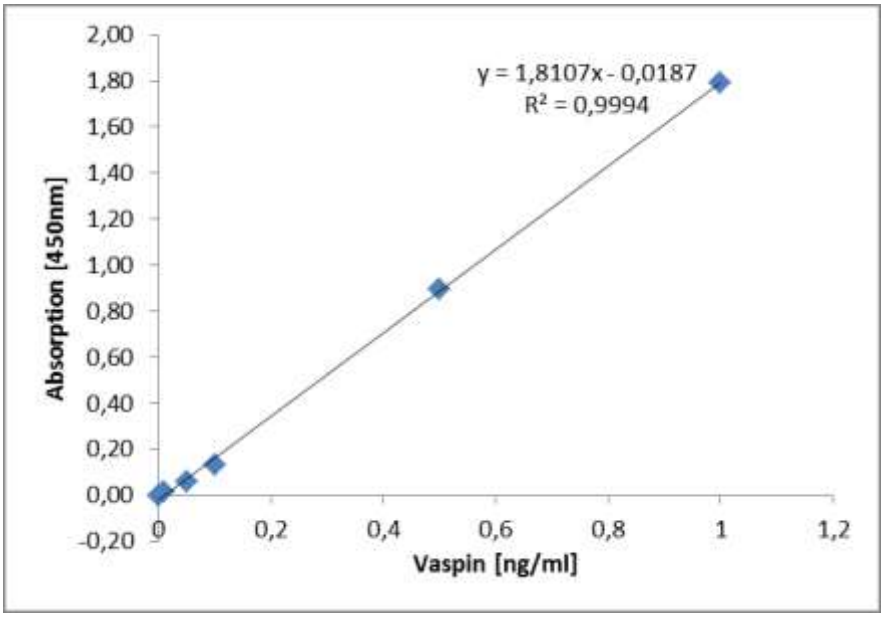


Fig. 1: Exemplary Standard Curve with linear regression for curve analysis.

The exemplary shown calibration curve in Fig.1 **cannot** be used for calculation of your test results. You have to establish a standard curve for each test you conduct!

Exemplary calculation of the Vaspin concentration of a 1:4 diluted sample:

Measured extinction of your sample (OD_{450}-OD_{620}) 0.56
Measured extinction of the blank (OD_{450}-OD_{620}) 0.03

Your measurement program will calculate the Vaspin concentration of the diluted sample automatically by using the difference of sample and blank (0.03) for the calculation. You only have to determine the most suitable curve fit (here: linear regression).

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

$0.53 = 1.8107x - 0.0187$

$0.303 = x$

If the dilution factor (1:4) is taken into account, the Vaspin concentration of the undiluted sample is
$0.303 \times 4 = 1.212$ ng/ml

PERFORMANCE CHARACTERISTICS

Standards

The standards are prepared from recombinant human Vaspin in concentrations of 10; 75; 200; 500 and 1000 pg/ml (equal to 0.010 -1 ng/ml).

Sensitivity

We measured the blank (dilution buffer only) 16 times in one assay. The resulting standard deviation was used for calculating the concentration which can be differentiated from the blank. Thus, lowest amount of Vaspin detectable is 4 pg/ml.

Table 1: Limit of Detection and Quantification

Standard Deviation [a.u.]	1 SD Vaspin [ng/ml]	3SD Vaspin [ng/ml]	10SD Vaspin [ng/ml]
0.015	0.004	0.012	0.037

Specificity

Specificity of the employed polyclonal antibodies was investigated by two different methods. Proteins of a native serum sample were separated by size exclusion chromatography, resulting fractions were tested a) by the ELISA and b) fractions positive in the ELISA were also tested by western blot. Figure 2 demonstrates that the ELISA detects a signal at about 37 kDa which is confirmed by western blot. Theoretically Vaspin has a molecular weight of 45.2 kDa. In comparison with recombinant Vaspin the signal of the SEC peak fraction is of the same size. Differences of the calculated molecular weight might result of the variability in column calibration as well as of the SEC conditions, like fraction size and flow velocity.

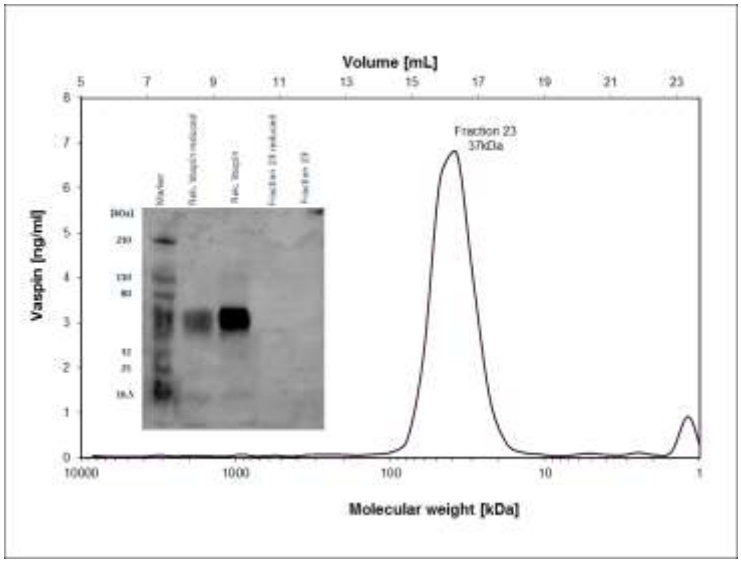


Fig. 2: Specificity of Mediagnost Vaspin ELISA. Human Serum (250 µL) was separated by SEC Superdex 10/300GL, fraction size was 0.5 ml and flow velocity 0.5 ml/min. Samples were diluted 1:4 in dilution buffer. Inset shows results of western blotting, samples were separated by 10% SDS-PAGE blotted and stained by biotinylated antibody (1:1000) and streptavidin-peroxidase conjugate (1:1500).

Recovery

For evaluation of disturbing substances in serum samples as well as assessing correctness we enriched several human serum samples with two different amounts of recombinant Vaspin. For control purposes same amounts of Vaspin were added to buffer and relative recovery in serum samples was calculated based on the value found in buffer.

Table 2: Recovery of recombinant Vaspin in human serum samples.

	Endogenous Vaspin content [ng/ml]	**Serum enriched with 50 pg/ml** [ng/ml]	**Serum enriched with 500 pg/ml** [ng/ml]
Recombinant Vaspin in Buffe**r**		0.052	0.408
Sample 1	0.233	0.271	0.591
Recovery [%]		**95**	**87**
Sample 2	0.1055	0.139	0.455
Recovery [%]		**88**	**89**
Sample 8	0.2595	0.302	0.705
Recovery [%]		**97**	**99**
Sample 9	0.517	0.588	1.013
Recovery [%]		**103**	**102**
Sample 4	0.118	0.151	0.432
Recovery [%]		**89**	**77**
Sample 5	0.744	0.943	1.066
Recovery [%]		**118**	**79**
Sample 17	1.457	1.601	1.915
Recovery [%]		**106**	**95**
Sample 18	0.2895	0.345	0.695
Recovery [%]		**101**	**92**

Recovery range was 77-102% for 500 pg/ml and 88 – 118% for 50 pg/ml enrichment, on avarage **97%** of the added recombinant material was found.

Reproducibility and Precision

Table 3: Inter-Assay-Variation (results of 3 independent determinations)

	Number	Mean [ng/mL]	VC%
Serum 1	9	0.189	8.55
Serum 2	9	0.823	4.52
Serum 3	9	2.885	4.75

Table 4: Intra-Assay-Variation

	Number	Mean [ng/mL]	VC%
Serum 1	20	0.226	2.85
Serum 2	20	1.192	3.81
Serum 3	20	1.536	1.42

Linearity

The linearity of serum dilutions is over a very wide range excellent. Two serum samples with high and low Vaspin content were serial diluted and dilution was measured by Vaspin ELISA. Results are shown In Figure 3. It was possible to quantify Vaspin down to 4 pg/ml. In both cases dilution resulted in linear decrease of concentration.

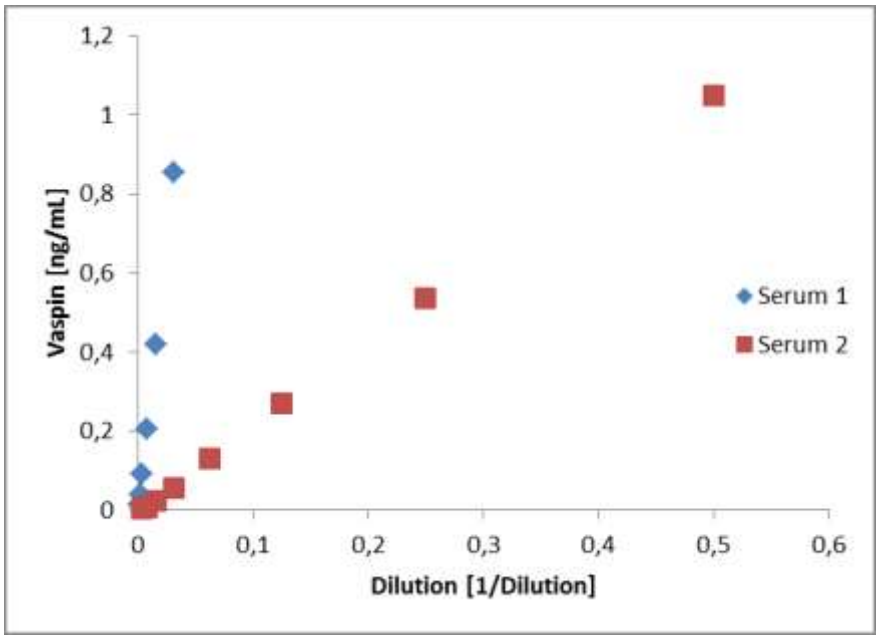


Fig. 3: Linearity. Two serum samples were diluted and concentration of each dilution was measured. Here concentrations are shown.

Assay Range

If no extreme values are expected, the dilution of 1:4 with Dilution Buffer VP is for most determinations suitable, the respective covered range would be 4 pg/ml- 4000 pg/ml.

Correlation with another test system.

Mediagnost Vaspin ELISA was compared with an commercially available Vaspin ELISA. We found a very good correlation of measured values even absolute values differ by factor 2 because of differences in calibration (Figure 4).

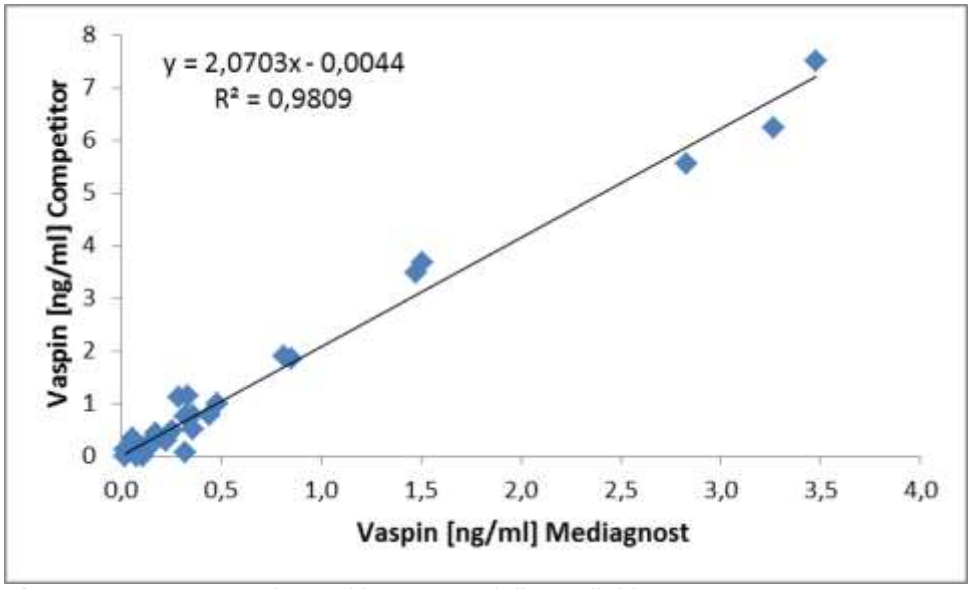


Fig. 4.: Assay Comparison with commercially available Testsystem

Reference values

Serum samples of a small group of healthy blood donors were used to assess the Vaspin concentration in healthy adults. No information regarding sampling time or contraceptive status was available. Samples were diluted 1:4.

Age [years]	Mean Males [ng/ml]	SD [ng/ml]	Number	Mean Femals [ng/ml]	SD [ng/ml]	Number
19-25	0.208	0.12	13	2.277	1.8	9
26-30	0.241	0.23	11	1.130	0.88	5
34-44	0.241	0.15	8	0.999	0.78	10
45-54	0.253	0.18	8	0.708	0.69	9
55-68	0.2402	0.20	5	0.503	0.31	5

In females an age-dependent decline of Vaspin concentrations might be apparent, but it should be reflected that oral contraceptives result in an increase of Vaspin serum concentration. Thus, instead of age dependent changes decline of Vaspin concentration might reflect a changed behavior of women correlating with age.

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SUMMARY Mediagnost VASPIN ELISA E106

Reconstitution / Dilution of Reagents		
Standards A-E	Reconstitution in **Dilution Buffer VP**	**750 µl each**
Control Serum KS1 & KS2	Reconstitution in **Dilution Buffer VP**	**250 µl each**
Washing Buffer WP	dilute in **A. dest.** (e.g. add the complete contents of the flask 50 ml into a graduated flask and fill with A.dest. to 1000 ml)	**1:20**
Sample Dilution + Control Sera KS1 & KS2: 1:4 in Dilution Buffer VP, **mix directly and use within max. 60 min.** Use **100 µl per determination**		
Before assay procedure bring all **reagents** to **room temperature**		

Proposal of Assay Procedure for Double Determination:

Pipette	**Reagents**	**Well Positions**
100 µl	Blank (**VP**)	A1 and A2
100 µl	Standard **A** (**10 pg/ml**)	B1 and B2
100 µl	Standard **B** (**75 pg/ml**)	C1 and C2
100 µl	Standard **C** (**200 pg/ml**)	D1 and D2
100 µl	Standard **D** (**500 pg/ml**)	E1 and E2
100 µl	Standard **E** (**1000 pg/ml**)	F1 and F2
100 µl	Diluted Control Serum **KS1 (1:4)**	G1 and G2
100 µl	Diluted Control Serum **KS2 (1:4)**	H1 and H2
100 µl	**Diluted Samples (1:4)**	Pipette sample in the rest of the wells according to requirements
Cover the wells with the sealing tape		

Incubation: **1 h at RT (20-25°C), 350 rpm**

5 x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP/well**	each well
100 µl	Antibody- Conjugate **AK**	each well

Incubation: **1 h** at **RT (20-25°C), 350 rpm**

5 x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP/well**	each well
100 µl	Enzyme-Conjugate **EK**	each well

Incubation: 1 h at RT (20-25°C), 350 rpm

5 x 300 µl	Aspirate the contents of the wells and wash **5x** with **300 µl** each **WP/well**	each well
100 µl	Substrate Solution **S**	each well

Incubation: 0.5 h in the dark at RT (20-25°C)

100 µl	Stop Solution **SL**	each well
Measure the absorbance within 30 min at **450 nm** (≥590 nm Reference)		

REF E106 **International Test Description**

STD A-E	**A -E**	**Rec in** 750 µl **BUF VP**	
Control	**KS1 / KS2**	**Rec in** 250 µl **BUF VP**	
WASHBUF 20x	**WP**		**1:20 DILU A. dest.**

Control	**1:4 DILU BUF VP**
SPE	**1:4 DILU BUF VP**
°C 20-25 °C	

100 µl	**BUF VP**	A1/2
100 µl	**STD A (10 pg/ml)**	B1/2
100 µl	**STD B (75 pg/ml)**	C1/2
100 µl	**STD C (200 pg/ml)**	D1/2
100 µl	**STD D (500 pg/ml)**	E1/2
100 µl	**STD E (1000 pg/ml)**	F1/2
100 µl	**CONTROL KS1 1:4 DILU BUF VP**	G1/2
100 µl	**CONTROL KS2 1:4 DILU BUF VP**	H1/2
100 µl	**SPE 1:4 DILU BUF VP**	
TAPE		

1 h °C 20-25 ↔ 350 rpm

5x 300 µl	**5x WASHBUF WP**
100 µl	**CONJ AK**
TAPE	

1 h °C 20-25 ↔ 350 rpm

5x 300 µl	**5x WASHBUF WP**
100 µl	**CONJ EK**

1 h °C 20-25 ↔ 350 rpm

5x 300 µl	**5x WASHBUF WP**
100 µl	**SUBST TMB S**

0.5 h °C 20-25

100 µl	**H_2SO_4 SL**
	MEASURE