Anti-Pseudomonas aeruginosa IgG ELISA

Enzyme Immunoassay for qualitative and quantitative Determination of

Human antibodies against Pseudomonas aeruginosa

English

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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

MTP AP, ELA, EXO

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

SPE

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

CONJ

KK Conjugate Concentrate / Konjugatkonzentrat/ conjugué conc / Coniugato concentrato / Conjugado concentrado / Conjugado Concentrado / conjugaat geconcentreerd / konjugat koncentrat/ konjugat koncentrat/ konjugat koncentrat / Koniugat koncentrátum / Antitest és enzim páros/ konjugát/ конюгат Концентрат / konjugaat kontsentraat / Σύμπλοκο αντισώματος-ενζύμου/ Concentrat Compuşi / Koncentrat konjugat / konjugaattitiiviste

DILU **X**

VP 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

Control

KS 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

Control POS

PK1, PK2, PK3 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 +

Control NEG	NK	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

Read entire protocol before use!

TECHNICAL FEATURES

- **For research use only!**
- Detects Antibodies against three Antigens Alkaline Protease, Exotoxin A and Elastase
- high sensitivity and specificity
- low intra- and interassay variability

INTRODUCTION

Pseudomonas aeruginosa, a Gram-negative bacterium ubiquitously distributed in the moist environment, causes about 10% of all nosocomial infections. This opportunistic pathogen leads to acute and chronic types of infection within the various organs.

P. aeruginosa infection provokes a rapid production of antibodies to a large number of *P. aeruginosa* antigens. **Mediagnost's** sensitive antibody detection system detects a *P.aeruginosa* infection very early at the onset.

Due to the use of three *P.aeruginosa* antigens, which are highly immunogenic and present in different parts from nearly all *P.aeruginosa* strains.

Depending on the *Pseudomonas aeruginosa* species and the immune reaction, antibodies can be detected against a single, two or even all three antigens simultaneously. A sample is regarded as sero-positive when antibodies against one or more of the antigens can be detected.

TEST PRINCIPLE

The **Mediagnost anti-*Pseudomonas aeruginosa* IgG anti-*Pseudomonas aeruginosa* IgG EIA, E15,** is a sandwich enzyme immunoassay. Serum or plasma samples are diluted and added to the wells of a microtiter plate, which have been previously coated with the *Pseudomonas aeruginosa* antigens alkaline protease, elastase or exotoxin A. Specific antibodies in the sample bind to the antigens present during an incubation of 2h at 37°C. After washing, the conjugate (anti-human IgG peroxidase-labelled immunoglobulin) is added and incubated again (for 2 h at 37°C). After a final washing step, substrate is added and further incubated for 30 min at room temperature. The reaction is terminated on addition of stop solution accompanied by a change from blue to yellow. The absorbance of the coloured reaction product is measured on a microtiter plate reader. The colour intensity of the reaction corresponds to the concentration of antibodies in the sample.

INTENDED USE

The Mediagnost anti-Pseudomonas aeruginosa IgG EIA, E15, is an enzyme immunoassay for **research use** and detects qualitatively and semi-quantitativley IgG-Antibodies in human serum against the extracellular proteins: **Alkaline Protease (AP), Elastase (Ela)** and **Exotoxin A (Exo)** of ***Pseudomonas aeruginosa*.**

PERFORMANCE CHARACTERISTICS AND VALIDATION

Table 1 : Linearity

Dilution	Sample		
	AP	**Ela**	**Exo**
1:750	1445		3623
1:1000	1429		3846
1:1500	1371		4123
1:3000	1162	10893	4230
1:6000		11135	4375
1:12000		10905	4590

Table 2 : Inter-Assay-Variation

	Number	Mean (Titer)			Standard deviation			Coefficient of Variation (%)		
		AP	**ELA**	**Exo**	**AP**	**ELA**	**Exo**	**AP**	**ELA**	**Exo**
Sample 1	74	1041	958	806	66	60	76	6	6	9
Sample 2	73	1516	1662	1167	77	135	74	5	8	6
Sample 3	72	3031	4129	4566	147	306	303	5	7	7

Table 3: Intra-Assay-Variation

	Number	Mean (Titer)			Standard deviation			Coefficient of Variation (%)		
		AP	**ELA**	**Exo**	**AP**	**ELA**	**Exo**	**AP**	**ELA**	**Exo**
Sample 1	16	3858	5521	5673	173	135	127	4.47	2.45	2.25
Sample 2	16	1453	1530	1381	88	44	142	6.08	2.86	10.29

SPECIMEN COLLECTION, PREPARATION AND STORAGE

Serum samples are suitable. A special external sample preparation prior to assay is not required. Slight hemolysis of the samples doesn't disturb the determination.

Samples should be handled as recommended in general: as fast as possible and chilled as soon as possible. In case there will be a longer period between the sample withdrawal and determination, store the undiluted samples frozen -20°C or below in tightly closable plastic tubes. Avoid on principal repeated freeze-thaw cycles of serum/plasma (if required, please subaliquote).

In most determinations (e.g. Serum or Plasma samples and no extreme values expected) the dilution of **1:1000 with Dilution Buffer VP is suitable.**
If the extinction of a sample exceeds the extinction of the positive control **PK** measurement should be repeated with the more diluted sample. For valid titer results samples should be measured in three dilutions (1:1000, 10000 and 100000).
Suggested Dilution Protocol:
Pipette 90 µl **Dilution Buffer VP** in PE-/PP-tubes (application of a multi-stepper is recommended in larger series), add **10 µl Serum**- or **Plasma** (dilution 1:10) and mix each tube.
In a second dilution step the pre-diluted samples should be diluted serial as follows:

10µl (1:10) Dilution in 990µl **VP** 1:1000
100µl (1:1000) Dilution in 900µl **VP** 1:10000
100µl (1:10000) Dilution in 900µl **VP** 1:100000

After mixing use **100 µl** of this solution within 1 hour **per determination** in the assay.

REAGENTS PROVIDED

1)	MTP AP	**Microtiter plate,** ready for use: Microtiter plate with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with alkaline Protease and **labelled red**, packed in a laminate bag.
2)	MTP ELA	**Microtiter plate,** ready for use: Microtiter plate with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with Elastase and **labelled blue**, packed in a laminate bag.
3)	MTP EXO A	**Microtiter plate,** ready for use: Microtiter plate with 96 wells, divided up in 12 strips with 8 wells separately breakable, coated with Exotoxin A and **labelled green**, packed in a laminate bag.
4)	BUF VP	**Dilution Buffer VP**, 3 × 50 ml, ready-to-use, please use for the dilution of the conjugate concentrate **KK** as well as for the dilution of **samples**
5)	Control POS PK1	**Positive Control PK1**, 1.5 ml, ready-to-use, calibrated control serum for alkaline Protease **AP red labelled** shows a titer of 1:2500
6)	Control POS PK2	**Positive Control PK2**, 1.5 ml, ready-to-use, calibrated control serum for Elastase **Ela blue labelled** shows a titer of 1:2500
7)	Control POS PK3	**Positive Control PK3**, 1.5 ml, ready-to-use, calibrated control serum for Exotoxin A **Exo green labelled** shows a titer of 1:2500
8)	Control NEG NK	**Negative Control NK**, 2 × 1.5 ml, ready-to-use, contains human serum, not reactive for Pseudomonas aeruginosa antigens
9)	Control	**Control Serum KS**, 2 × 1.5 ml**,** ready-to-use, contains human serum and has to be determinated as borderline in the assay
10)	CONJ	**Conjugate Concentrate KK**, 2 × 250 µl, 100fold Concentrate, contains POD (horseradish-Peroxidase)-labelled anti-human IgG. After dilution the Cojugate is only limited stable, dilute only according to requirements.
11)	WASHBUF 20x	**Washing Buffer (WP),** 120 ml, 20 X concentrated solution. **Washing Buffer (WP)** has to be diluted 1:20 with distilled or demineralised water before use. Attention: After dilution the Washing Buffer is only 4 weeks stable, dilute only according to requirements.
12)	SUBST	**Substrate (S),** 33 ml, ready for use, horseradish-peroxidase-(HRP)-substrate, stabilised H_2O_2 Tetramethylbencidine.
13)	H_2SO_4	**Stopping Solution (SL),** 33 ml, ready for use, 0.2 M sulphuric acid, Caution acid!
14)		Sealing tape for covering of the microtiter plate, 6 x, adhesive.

MATERIALS REQUIRED BUT NOT PROVIDED

Precision pipettes, Micropipettes and multichannel pipettes with disposable plastic tips
Distilled or deionized water for dilution of the Washing Buffer (WP)
Vortex-mixer
Microtiter plate washer (recommended)
Micro plate reader ("ELISA-Reader") with filter for 450 and ≥590 nm
Polyethylen PE/Polypropylen PP tubes for dilution of samples

TECHNICAL NOTES

In conducting the assay, follow strictly the test protocol. Room temperature incubation means: Incubation at 20 - 25°C.

Reagents with different lot numbers should not be mixed. The microtiter plate and all reagents are stable unopened until the expiry date, if stored in the dark at 2° - 8°C (see label).

The shelf life of the components after opening is not affected, if used appropriately. Store the unused stripes of the microtiter plate together with the desiccant airtight at 2-8°C.

Use the **Dilution Buffer VP** for the dilution of **Conjugate Concentrate KK.**

The 1:20 diluted Washing Buffer **WP** is only 4 weeks stable. Please dilute only according to daily requirements. This applies to the 1:100 diluted **Conjugate Concentrate KK** too.
Before use, all kit components should be brought to room temperature. **Precipitates, possible in buffers, should be dissolved before use through mixing and warming**.

Incubation at room temperature means: Incubation at 20-25°C

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 dynamicly 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.

The **Substrate Solution S**, stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.
When performing the assay, Controls **KS, PK, NK** and the samples should be pipetted as fast as possible (e.g., 15 minutes). To avoid distortions due to differences in incubation times the diluted Conjugate Concentrate **KK** 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**

STORAGE CONDITIONS

The microtiter plate wells and all undiluted reagents are stable until the expiry date if stored in the dark at 2-8°C.
Store the unused microtiter wells together with the desiccant at 2° to 8°C.
The shelf life of the components after opening is not affected, if used appropriately.
The Substrate Solution (S), stabilised H_2O_2-Tetramethylbencidine, is photosensitive – store and incubate in the dark.

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.

Before use, all kit components should be brought **to room temperature at 20 - 25°C**. Precipitates in buffers should be dissolved before use by thorough mixing and warming. **Temperature WILL affect the absorbance** readings of the assay. However, Values for the samples will not be affected.

Do not mix reagents of different lots. Do not use expired reagents.

The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.

Caution: This kit contains material of human and/or animal origin. Source human serum for the Control Serum provided in this kit was tested by FDA recommended methods 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 specimens should be treated as potentially infectious.

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.

Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step. Use separate pipette tips for each sample, control and reagent to avoid cross contamination. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution

colored. Do not pour reagents back into vials as reagent contamination may occur. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.

Following components contain **0.01% 2-Methyl-4-isothiazolin-3-one Solution** as preservative: **KK, VP**

R34	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S36/37	Wear suitable protective clothing and gloves
S45	In case of accident or if you feel unwell seek medical advice

Following components contain **0.01% (w/w) 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-isothiazol-3-**one as preservative: **KK, VP, WP**

R36/38	Irritating to eyes and skin
R43	Sensibilisation through skin contact possible
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

TMB-Substrate (S) contains 3,3´,5,5´ Tetramethylbenzidine.

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

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.
Do not eat, drink or smoke in these areas.
Never pipette the materials with the mouth.
Spilled material must be wiped off immediately and should become disinfected. Clean contaminated areas and equipment with a suitable detergent.

ASSAY PROCEDURE

NOTES: All determinations (Controls and samples) should be assayed in duplicate. For optimal results, accurate pipetting and adherence to the protocol are recommended.
When performing the assay, the Positive Control, Negative Control, Control Serum and the samples should be pipette as fast as possible (e.g., <15 minutes). To avoid distortions due to differences in incubation times, the diluted **conjugate concentrate KK** as well as the following **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.

1) pipette in positions* A1/2 **100 µl Negative Control NK each**
2) pipette in positions* B1/2 **100 µl Positive Control PK each** (PK1,PK2 OR PK3, respectively)
3) pipette in positions*C1/2 **100 µl Control Serum KS each**
   * of Microtiter plates PL1/PL2/P3
4) Pipette **100 µl each** of the **diluted sample** (generally 1:1000 diluted in Dilution Buffer **VP**) in the rest of the wells, according to requirements
5) Cover the wells with the sealing tape and incubate the plate for **2 hours** at **37°C**
6) After incubation aspirate the contents of the wells and wash the wells 3 times with **300 µl Washing Buffer WP**. Empty the wells thoroughly.
7) Following the last washing step, pipette **100 µl** of the **1:100 diluted Conjugate Concentrate KK** in each well.
8) Cover the wells with the sealing tape and incubate the plates for **2 hours** at **37°C**.
9) After incubation wash the wells 3 times with **Washing Buffer WP** as described in step 6)
10) Pipette **100 µl of the TMB-Substrate solution S** in each well.
11) Incubate the plates for **30 Minutes in the dark at** room **temperature**.
12) After incubation pipette **100 µl Stop Solution SL** in each well.

Measure the absorbance **within 30 minutes at 450 nm (Reference filter ≥590 nm)**

CALCULATION OF RESULTS

Calculations should be performed for each antigen (each plate) separately! Calculate the average of all multiple values.

For the evaluation of the assay it is preconditioned that the absorbance values of the Negative Controls NK should be below 0.25. The difference between the extinctions of Negative Controls NK and the respective Positive Controls PK must be at least 0.6.

Qualitative Calculation:

The negative control average is subtracted from the controls and samples to obtain absolute values (Blank).

Cut-off calculation:
The cut-off is 20% of the positive control average.

This value corresponds to a titre of 1:500 of a 1:1.000 diluted serum. 1:1.000 diluted sera with extinction values less than the cut off are regarded as negative.

Depending on the *Pseudomonas aeruginosa* species and the immune reaction, antibodies can be detected against a single, two or even all three antigens simultaneously. A sample is regarded as sero-positive when it is positive for one or more of the antigens.

Negative Control NK		**Extinction**
1.Value		0.041
2.Value		0.056
Mean		**0.049**

Positive Control PK		**Extinction**
1.Value		1.120
2.Value		1.136
Mean		**1.128**

Calculation

PK – NK	: 1.128 – 0.049	= 1.079
Cut-off (20% of PK-NK)	: 0.2 × 1.079	= 0.216
Borderline (50% of PK-NK)	: 0.5 × 1.079	= 0.540

All samples with an extinction < 0.216 are determined as negative for anti-P. aeruginosa IgG. Samples with an extinction of > 0.216 and < 0.540 are judged as borderline and samples showing an extinction of > 0.540 are determined as positive for anti-P. aeruginosa IgG.

Semi-quantitative calculation

The measured extinction values of the individual serum dilutions are plotted onto a graph or calculated using an appropriate computer programme.

The extinction values of the negative (NK) and positive (PK) controls are plotted on the y-axis of a linear plot. A so-called titre factor is in turn plotted on the x-axis, for NK = 0 and PK = 2.5. For the quantification of the sera values, a straight line is drawn through the NK and PK values and extended to a value of 3.5.
The titre of the individual serum is determined by reading the **titre factor** of the measured extinction value through the NK-PK axis, which is multiplied by the serum dilution factor.
Titre factors lower than 0.25 and higher than 3.5 (x-axis) are not taken into consideration in these calculations, except that the titre factors from a 1:1000 dilution below 0,5 are regarded as negative and titre factors greater than 3,5 are always positive and should be diluted further and retested for quantitative determination.

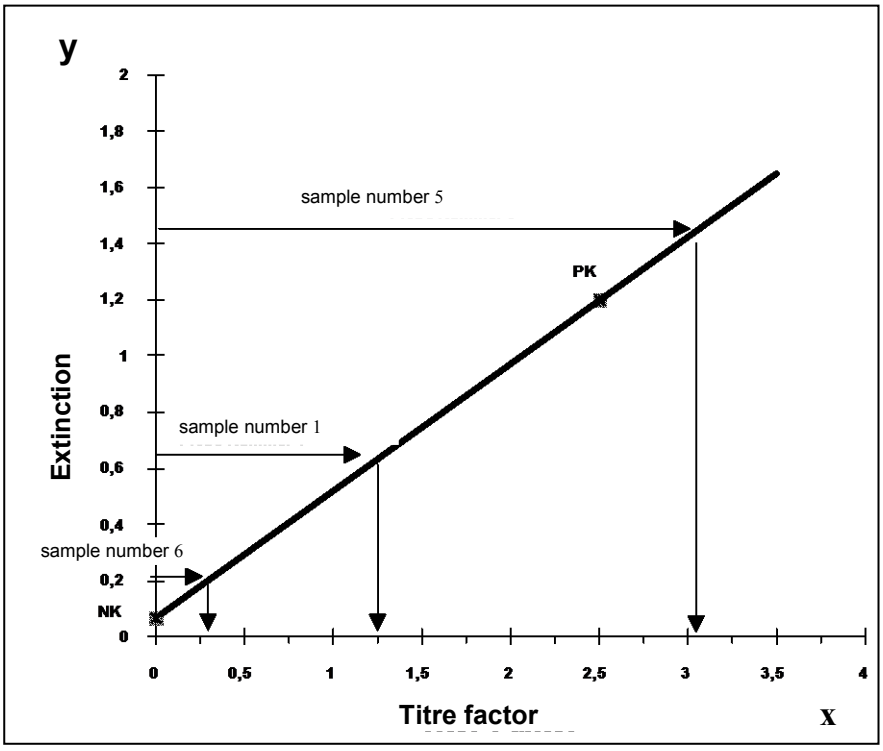
Categories:
The semi-quantitative detection of human sera using the **mediagnost anti-*Pseudomonas aeruginosa* IgG EIA , E15,** is subdivided into the following categories:

Titre	Interpretation
< 1:500	negative
1:500 to 1:1 250	border-line
> 1:1 250	positive
> 1:10 000	chronically positive

Negative Control NK		**Extinction**
1.Value		0.041
2.Value		0.056
Mean		**0.049**

Positive Control PK		**Extinction**
1.Value		1.220
2.Value		1.176
Mean		**1.198**

Graphic



Calculation

Sample No.	Sample Dilution	Extinction (average)	Titre factor (see graphic)	Titre	Interpretation
Example:	Serum border-line				
1	Serum A 1:1 000	0.625	1.25	1:(1 000 x 1.25) => 1: 1 250	border-line
2	Serum A 1:10 000	0.075	< 0.25		cannot be calculated
3	Serum A 1:100 000	0.05	< 0.25		cannot be calculated
Example	Serum positive				
4	Serum B 1:1 000	2.82	> 3.5		positive
5	Serum B 1:10 000	1.45	3.05	1:(10 000 x 3.05) =>1:30 500	chronically positive
6	Serum B 1:100 000	0.210	0.3	1:(100 000 x 0.3) => 1:30 000	chronically positive
Example	Serum negative				
7	Serum C 1:1000	0.064	< 0.25	< 1:500	negative

LITREATURE / LITERATUR

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SUMMARY – MEDIAGNOST P. aerugionsa IgG EIA E15

Dilution of Reagents and Samples		
Conjugate concentrate KK	**in Dilution Buffer VP**	**1:100**
Washing buffer WP	**in Aqua dest**. (e.g. add 100 ml **WP** in to a graduated flask and fill with **A.dest** to 2000 ml)	**1:20**
Dilute samples **1:1000** with dilution buffer (qualitative test). For quantitative antibody assays it is recommended to perform dilutions of **1:1.000**, **1:10.000** and **1:100.000**.		

Proposal of Assay Procedure for double determinations

Pipette	**Reagents**	**Plate 1 AP**	**Plate 2 Ela**	**Plate 3 Exo**
2 x 100 µl	Negative Control **NK**	A1/A2	A1/A2	A1/A2
2 x 100 µl	Positive Control **PK1**	B1/B2		
2 x 100 µl	Positive Control **PK2**		B1/B2	
2 x 100 µl	Positive Control **PK3**			B1/B2
2 x 100 µl	Control Serum **KS**	C1/C2	C1/C2	C1/C2
2 x 100 µl	Sample dilution	Pipette in the rest of the wells according to requirements.		
Seal the wells with the seeling tape				

Incubation: 2 h at 37°C

3 x 300 µl	Aspirate the contents of the wells and **wash 3x** with **300 µl** each Wash Buffer **WP/ well**	in each well
100 µl	1:100 diluted **Conjugate concentrate KK**	in each well
Seal the wells with the seeling tape		

Incubation: 2 h at 37°C

3 x 300 µl	Aspirate the contents of the wells and **wash 3x** with **300 µl** each Wash Buffer **WP/ well**	in each well
100 µl	Substrate Solution **S**	in each well

Incubation: 30 min in the dark at RT

100 µl	Stopping Solution **SL**	in each well
Measure the absorbance within **30 min** at **450 nm** with **≥ 590 nm** as reference wavelength.		

International Test description

CONJ	**KK**	**1:100 DILU BUF VP**	
WASHBUF 20x	**WP**		**1:20 DILU A. dest.**

SPE 1:1000 DILU BUF VP ↔	
°C 20-25 °C	

		MTP AP	**MTP ELA**	**MTP Exo**
100 µl	**Control NEG NK**	A1/2	A1/2	A1/2
100 µl	**Control POS PK1**	B1/2		
100 µl	**Control POS PK2**		B1/2	
100 µl	**Control POS PK3**			B1/2
100µl	**CONTROL KS**	C1/2	C1/2	C1/2
100 µl	**SPE 1:1000 DILU BUF VP**			
TAPE				

2 h °C 37

3x 300 µl	**3x WASHBUF WP**
100 µl	**CONJ KK 1:100 DILU VP**
TAPE	

2 h °C 37

3x 300 µl	**3x WASHBUF WP**
100 µl	**SUBST TMB S**

0.5 h °C 20-25 

100 µl	**H_2SO_4 SL**
	MEASURE