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STATEMENT

We, D**IALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.**, having a registered office at **IZ-NOE Sued Hondastrasse, Objekt M55, A-2351 Wr. Neudorf, AUSTRIA** assign SRL SANMEDICO having a registered office at A. Corobceanu street 7A, apt. 9, Chişinău MD-2012, Moldova , as authorized representative in correspondence with the conditions of directive 98/79/EEC. We declare that the company mentioned above is authorized to register, notify, renew or modify the registration of medical devices on the territory of the Republic of Moldova. This declaration will stay in force for 2 years or if one of the parties is deciding to cancel it with a one-month notice.

Date :05.04.2023
Signature:

DIALAB
Produktion und Vertrieb von chemisch - technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
A - 2351 Wr. Neudorf, IZ-NO Süd, Hondastr. Obj.M55
Phone: ++43 (0)2236 660910 - 0 Fax ++43 (0) 2236 660910 - 30
E-Mail: office@dialab.at Website: www.dialab.at

Digitally signed by Monastîrschii Viorica
Date: 2023.12.14 15:42:50 EET
Reason: MoldSign Signature
Location: Moldova

Christina Ernst
Export Manager

EC DECLARATION OF CONFORMITY

EG-KONFORMITÄTSERKLÄRUNG

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IZ-NOE Sued, Hondastrasse, Objekt M55, A-2351 Wiener Neudorf

REF	Product Name / Produktname	Content / Inhalt
R97413	Anti-Cardiolipin IgG/IgM	96 wells

We declare, on our own responsibility, that our above-mentioned product classified as follows according to the directive on in vitro diagnostic medical devices 98/79/EC:
Devices other than those covered by Annex II

meets the applicable provisions of the EU Directive 98/79/EC for in-vitro-diagnostic medical devices and the Austrian Medical Product Law.

This Declaration is based on the Conformity Assessment Procedure according to Annex III of the aforesaid Directive.

This Declaration is valid until 2025-03-28.

Hiermit erklären wir, auf eigene Verantwortung, dass unser oben genanntes Produkt, gemäß der Richtlinie 98/79/EG über In-vitro-Diagnostika klassifiziert als:
Andere als die in Anhang II genannten Produkte

die anwendbaren Vorschriften der EU-Richtlinie 98/79/EG über in-Vitro-Diagnostika und des Österreichischen Medizinproduktegesetzes erfüllt.

Diese Erklärung basiert auf dem Konformitätsbewertungsverfahren nach Anhang III der oben angeführten Richtlinie.

Diese Erklärung ist bis zum 2025-03-28 gültig.

Produktion und Vertrieb von chemisch - technischen
Produkten und Laborinstrumenten Gesellschaft m.b.H.
A - 2351 Wr. Neudorf, IZ NÖ Süd, Hondastr. Obj.M55
Phone: ++43 (0) 2236 660910 - 0 Fax ++43 (0) 2236 660910 - 30
E-Mail: office@dialab.at Website: www.dialab.at

Wiener Neudorf, 2022-03-29

Heidi Kroiß
Qualitätsmanagementbeauftragte
Quality Management Representative

EC DECLARATION OF CONFORMITY

EG-KONFORMITÄTSERKLÄRUNG

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IZ-NOE Sued, Hondastrasse, Objekt M55, A-2351 Wiener Neudorf

REF	Product Name / Produktname	Content / Inhalt
R99449	Anti-Phospholipid Screen IgG/IgM	96 wells

We declare, on our own responsibility, that our above-mentioned product classified as follows according to the directive on in vitro diagnostic medical devices 98/79/EC:
Devices other than those covered by Annex II

meets the applicable provisions of the EU Directive 98/79/EC for in-vitro-diagnostic medical devices and the Austrian Medical Product Law.

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A - 2351 Wr. Neudorf, IZ-NÖ Süd, Hondastr. Obj.M55
Phone: ++43 (0) 2236 660910 - 0 Fax ++43 (0) 2236 660910 - 30
E-Mail: office@dialab.at Website: www.dialab.at

Wiener Neudorf, 2022-03-29

Heidi Kroiß
Qualitätsmanagementbeauftragte
Quality Management Representative

ZERTIFIKAT ◆ CERTIFICATE ◆ 認證證書 ◆ СЕРТИФИКАТ ◆ CERTIFICADO ◆ CERTIFICAT

DAkkS Deutsche Akkreditierungsstelle D-ZM-11321-01-00

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No. Q5 026709 0009 Rev. 01

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Certification Mark:

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Valid from: 2022-03-29
Valid until: 2025-03-28

Date, 2022-03-17

Christoph Dicks
Head of Certification/Notified Body

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Medical devices - Quality management systems - Requirements for regulatory purposes
(ISO 13485:2016)
DIN EN ISO 13485:2016

Facility(ies):

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ELISA **E**NZYME **L**INKED **I**MMUNO**S**ORBENT **A**SSAY

Microwell Method

Anti - Cardiolipin IgG/IgM

Cat. No. R97413

For in vitro Diagnostic Use

P r o d u c t I n s e r t

Enzyme Linked Immunosorbent Assay for the **quantitative** determination of IgG or IgM autoantibodies against Cardiolipin in human serum or plasma.

CE IVD

Microwell Method - 96 wells

(12 x 8-well Antigen coated Strips)

Individual breakaway

GENERAL INFORMATION

- **Wavelength**

  Measurement Filter: 450 nm

  Optional Reference Filter: 600 - 690 nm

- **Incubation Time**

  60 minutes at RT (30/15/15)

- **Enzyme Conjugate**

  HRP (Horseradish Peroxidase)

- **Substrate**

  TMB (3,3´,5,5´-Tetramethyl-benzidine)

- **Sample**

  Serum or Plasma

- **Stability of Samples**

  undiluted: 5 days at 2-8°C; up to 6 months at - 20 °C

- **Calibration Range**

  0 - 120 GPL U/ml / 0 - 80 MPL U/ml

- **Sensitivity**

  1 GPL U/ml / 0.5 MPL U/ml

  Indications

  - Thrombocytopenia
  - Cerebrovascular Insufficiency

KIT COMPONENTS

Microwell plate	12	**8 well strips** with breakaway microwells coated with highly purified bovine Cardiolipin and saturated with highly purified human ß2-Glycoprotein I, **ready to use.**
Positive Control	1	vial, 1.5 ml, **(red cap), ready to use.** see Quality Control Certificate for expected value and range (PBS, NaN_3 <0,1% (w/w)
Negative Control	1	vial, 1.5 ml **(blue cap), ready to use.** see Quality Control Certificate for expected value and range (PBS, NaN_3 <0,1% (w/w)
Calibrators combined for IgG and IgM class Ab	6	vials, each 1.5 ml, **ready to use.** IgG: 0; 7.5; 15; 30; 60; 120 GPL U/ml IgM: 0; 5; 10; 20; 40; 80 MPL U/ml
IgG-IgG Enzyme Conjugate	1	vial of polyclonal rabbit anti-h-IgG-IgG HRP conjugate, 15 ml, color coded **pink**, **ready to use**. (PBS, PROCLIN 300<0,5% (v/v)
IgM-IgG Enzyme Conjugate	1	vial of polyclonal rabbit anti-h-IgM-IgG HRP conjugate, 15 ml, color coded **pink**, **ready to use**. (PBS, PROCLIN 300<0,5% (v/v)
Substrate Solution	1	vial of TMB, 15 ml, **ready to use.**
Stop Solution	1	vial of phosphoric acid (<5%), 15 ml, **ready to use**.
Sample Diluent	1	vial, 20 ml, **concentrate (5x),** color coded **yellow.**
Wash Buffer	1	vial, 20 ml, **concentrate (50x).**

MATERIALS REQUIRED BUT NOT PROVIDED

- Deionized or distilled water
- Graduated cylinders and beakers
- Wash trays
- **Macropipettes** capable of delivering 5 µl to 1000 µl. We recommend Dialab Digital Macropipette. Please ask for Dialab Liquid Handling catalog.
- **Multichannel Micropipette**; we recommend Dialab Digital Multichannel Micropipette. Please ask for Dialab Liquid Handling catalog.
- **Stepper**; we recommend Dialab Stepper. Please ask for Dialab Liquid Handling catalog.
- **Microplate reader** capable of reading absorbance values at 450 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-690 nm.

- **Automatic microplate washer** capable of dispensing 300 µl.
  Microplate reader and microplate washers are available from Dialab Company.

SUMMARY AND EXPLANATION

Antiphospholipid antibodies are a heterogeneous group of autoantibodies against cardiolipin (diphoshatidyl glycerol) a negatively charged phospholipid[1]. They are detected primarily by the anti-cardiolipin antibody (ACA) test, the biological false positive test for syphilis and the lupus anticoagulant test. These three tests detect related, but not necessarily identical antibodies. Thus, more than one of these tests is sometimes necessary to identify antiphospholipid antibodies. It has been postulated that anti-phospholipid autoantibodies might be responsible for causing the blood clotting mechanism to loose its normal control. A possible mechanism for the formation of thromboembolism is the following:
Anti-cardiolipin Antibodies bind to phospholipids on endothelial cells lining blood vessels and tissues of the heart. Complement binds to the autoantibody-cardiolipin complexes causing inflammation and destruction of endothelial cells. Inflammation results in swelling around the blood vessel and small clots form. Clots can either block circulation locally or break off and block circulation elsewhere. This blockage of circulation is called an infarction.
The presence of $ß_2$-Glycoprotein is required for the binding of anti-Cardiolipin antibodies to cardiolipin. Recent studies have shown that anti-Cardiolipin antibodies either recognize cardiolipin on its own or the complex of cardiolipin and $ß_2$-GPI.

TEST PRINCIPLES

The ELISA test is performed as an indirect solid phase **sandwich-type** immunoassay. Microwells are coated with Cardiolipin antigen followed by blocking the unreacted sites to reduce non-specific binding.

Step 1	Antibodies specific to cardiolipin present in controls, calibrators and patient samples bind to the coated antigen.
Step 2	The Antigen-Antibody complex is reacted with enzyme (HRP) labeled anti-human-IgG or IgM conjugate resulting in the anti-cardiolipin antibody of the appropriate class being sandwiched between the solid phase antibody and the enzyme conjugate.
Step 3	The enzyme converts added substrate (TMB) to form a colored solution.
Step 4	The intensity of color change, which is proportional to the concentration of Antibodies present in the samples, is read by a microplate-reader at 450 nm. Results are expressed in GPL U per milliliter (GPL U/ml) or MPL U per milliliter (MPL U/ml).

EXPECTED VALUES

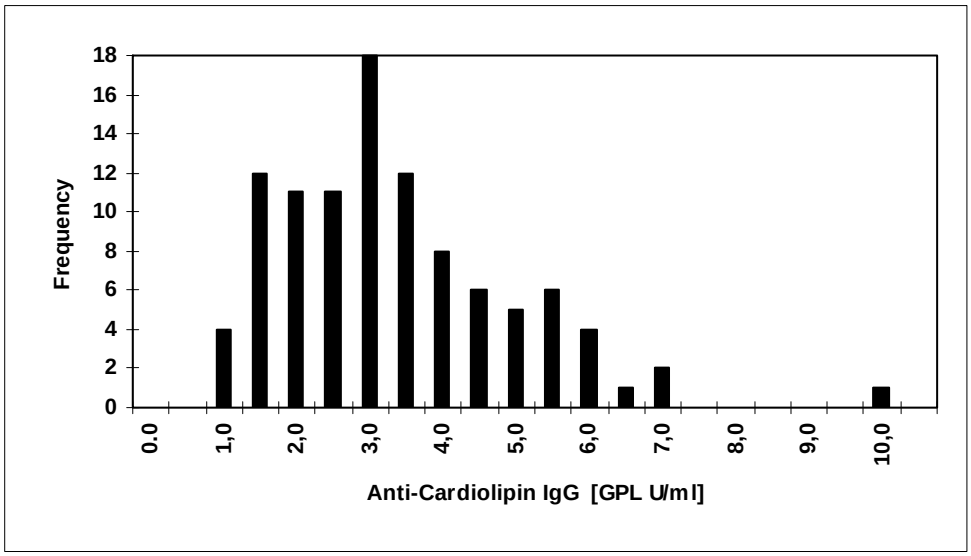
Normal Values

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the anti-Cardiolipin tests:

	anti-Cardiolipin-Ab	
	IgG [GPL U/ml]	IgM [MPL U/ml]
normal:	< 10	< 7
elevated:	> 10	> 7

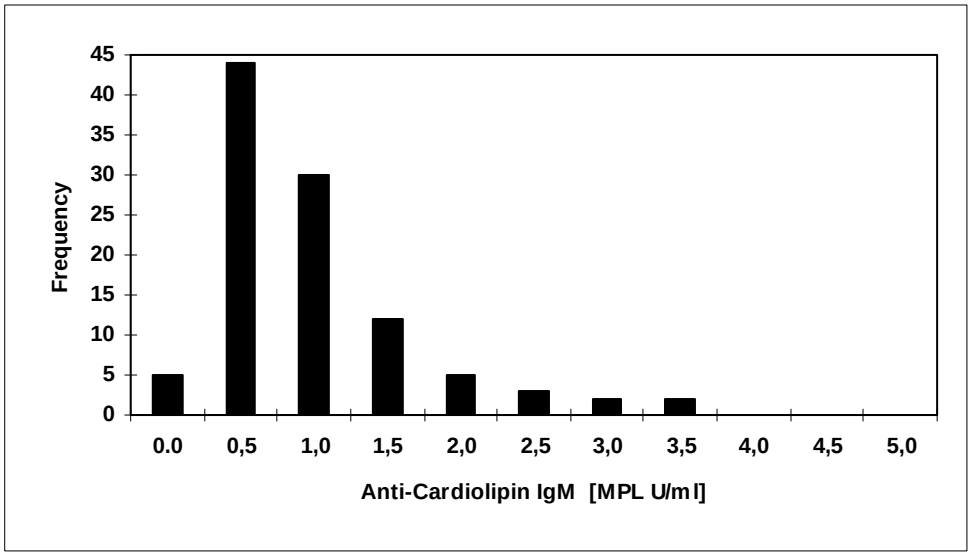
Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually.
It is recommended that each laboratory establishes its own normal and pathological ranges of serum anti-Cardiolipin. The values below should be regarded as guidelines only.

Frequency distribution of anti-Cardiolipin **IgG class antibodies** in healthy donors



mean ± 2SD = 3.4 ± 3.2 GPL U/ml, n = 101

Frequency distribution of **anti-Cardiolipin IgM class antibodies** in healthy donors



mean ± 2SD = 0.98 ± 1.42 MPL U/ml, n = 103

REAGENTS

Storage

- ❑ Store all reagents at 2° - 8°C. Do not freeze!

Preparation

- ❑ Coated microwell strips are for one time use only.
- ❑ Pos. Control, Neg. Control, Calibrators, Enzyme Conjugate, Substrate Solution and Stop Solution are ready to use and need not to be diluted.
- ❑ Wash Buffer and Sample Diluent are concentrated and need to be diluted (see page 7).

Precautions

- ❑ Instructions should be followed exactly as they appear in this kit insert to ensure valid results.
- ❑ Avoid contact with the **TMB (3,3`,5,5`-Tetramethyl-benzidine)**. If TMB comes into contact with skin wash thoroughly with water and soap.
- ❑ The stop solution contains **phosphoric acid**. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
- ❑ Avoid contact between the buffered **peroxide** solution and easily oxidized materials; extreme temperatures may initiate spontaneous combustion.
- ❑ Do not use beyond expiration date on the label.
- ❑ Do not use if reagent is not clear or if a precipitate is present.
- ❑ Do not interchange kit components with those from other sources other than the same catalog number from DIALAB.
- ❑ Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling.
- ❑ All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. However, human blood derivatives and patient specimens should be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials.

SPECIMEN COLLECTION AND HANDLING

- ❑ Only **Serum or Plasma** specimens should be used in this procedure. The patients need not to be fasting, and no special preparations are necessary.
- ❑ Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Neither Bilirubin nor Hemolysis have significant effect on the procedure.
- ❑ Store specimens at **2°- 8°C** for up to a maximum of **5 days**. For longer storage, specimens should be frozen. Avoid repeated freezing and thawing of samples.
- ❑ **Specimen preparation** see page 7.

PROCEDURE

Procedural Notes

- ❑ Before starting with the assay read carefully the product insert.
- ❑ Let specimens and test reagents equilibrate at room temperature before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- ❑ Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.
- ❑ All dilutions of patient samples should be prepared prior to starting with the assay.
- ❑ Good washing technique is critical. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. For automatic washers, program the washer as per manufacturer's instructions.
- ❑ Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- ❑ For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.

Preparation of Reagents

Sample Diluent concentrate (5x):	Dilute to a final volume of 100 ml with distilled water prior to use.
Wash Buffer concentrate (50x):	Dilute to a final volume of 1000 ml with distilled water prior to use.

Preparation of Sample Dilutions

Serum or Plasma Samples: Dilute 1:100 with sample diluent. For example:

10 µl serum or plasma	+ 990 µl sample diluent

Test Procedure

Step 1 Prepare Wash Buffer and Sample Diluent.

Step 2 Prepare Sample Dilutions.

Step 3 Label protocol sheet to indicate sample placement in the wells according to the following figure. **6 calibrators** (standards) (SA-SF), **2 Positive Controls** (PC) and **2 Negative Controls** (NC) should be included. The user has the option to run Patient Samples (P) in duplicate.

	1	2	3	4	5	6	7	8	9	10	11	12
a	SA	SE	P1									
b	SA	SE	P1									
c	SB	SF	P2									
d	SB	SF	P2									
e	SC	PC	P..									
f	SC	PC	P..									
g	SD	NC										
h	SD	NC										

Calibrator	IgG Conc. GPL U/ml	IgM Conc. MPL U/ml
SA	0	0
SB	7.5	5
SC	15	10
SD	30	20
SE	60	40
SF	120	80

Step 4 Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder.

Step 5 Pipette **100 µl** of **Calibrators, Controls and prediluted Patient Samples** into the wells.

Step 6 Incubate **30 minutes** at room temperature (20 – 28°C).

Step 7 Discard the contents of the microwells and wash **3x** with **300 µl** wash buffer. Minimum soaking time 25sec. Wash buffer must be removed completely from the wells. Therefore blot vigorously the empty plate upside down several times on adsorbent paper.

Step 8 Pipette **100 µl** of **Enzyme Conjugate (anti-h-IgG-IgG or anti-h-IgM-IgG)** into microwells.

Step 9 Incubate **15 minutes** at room temperature (20 – 28°C)

Step 10 Wash all microwells **3 x** as in Step 7.

Step 11 Pipette **100 µl of Substrate Solution** into each microwell in the same order and timing as for the Enzyme Conjugate.

Step 12 Incubate **15 minutes** at room temperature (20 – 28°C).

Step 13 Add **100 µl of Stop Solution** into each microwell using the same order and timing as for the addition of the Substrate Solution. Let stand for 5 minutes.

Step 14 Read absorbance of each microwell at **450 nm** using a single, or at **450/600-690 nm** using a dual wavelength microplate reader. The developed color is stable for at least 30 minutes. Read optical densities during this time.

Quality Control

One positive and one negative control should be included in duplicate in each assay and should be within range given for the Lot. No.

TEST EVALUATION

Manual Evaluation: recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator concentration. A **standard curve** is obtained from a lin-log plot. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the calibrator concentrations. Draw the best fitting curve approximating the path of all calibrator points. The unknown patient sample concentrations are determined by comparing the mean OD value for each sample to the standard curve and reading off the corresponding concentration.

Computer-assisted Evaluation: recommended 4-Parameter-Fit

For computer-assisted evaluation of anti-Cardiolipin IgG or IgM test a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is recommended. Smoothed Spline approximation and log-log coordinates are also suitable. Samples can be read directly from the standard curve.

LIMITATIONS OF THE PROCEDURE

The assay should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples. This method should be used for testing human serum samples only.

PERFORMANCE CHARACTERISTICS

Sensitivity

The lower detection limit for anti-Cardiolipin IgG was determined at 1.0 GPL U/ml.
Anti-Cardiolipin IgM yielded a sensitivity of 0.5 MPL U/ml.

Interferences

There are no interferences with hemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or sera with elevated Bilirubin (up to 40 mg/dL).

Parallelism

In dilution experiments sera with high IgG- and IgM-antibody concentrations were diluted with sample diluent and assayed in the anti-Cardiolipin kit.

Anti-Cardiolipin	Sample	Dilution	**O**bserved [U/ml]	**E**xpected [U/ml]	**O/E**
IgG	1	1:200	126.7		
		1:400	63.7	63.4	100 %
		1:800	32.9	31.7	104 %
		1:1600	14.1	15.8	89 %
		1:3200	7.2	7.9	91 %
IgG	2	1:100	112.3		
		1:200	56.1	56.2	100 %
		1:400	25.0	28.1	89 %
		1:800	12.0	14.0	86 %
		1:1600	6.0	7.0	86 %
IgM	3	1:100	55.0		
		1:200	27.0	27.5	98 %
		1:400	13.0	13.8	94 %
		1:800	6.4	6.9	93 %
IgM	4	1:200	46.5		
		1:400	23.2	23.3	100 %
		1:800	10.9	11.6	94 %
		1:1600	5.2	5.8	90 %
		1:3200	2.8	2.9	97 %

Precision

Statistics for Coefficients of variation (CV) were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations each:

anti-Cardiolipin (IgG)

Intra-Assay		
Sample No	Mean x [GPL U/ml]	CV [%]
1	29.1	5.4
2	62.5	5.8
3	109.4	4.1

Inter-Assay		
Sample No	Mean x [GPL U/ml]	CV [%]
1	32.9	3.8
2	70.9	2.5
3	118.3	2.7

anti-Cardiolipin (IgM)

Intra-Assay		
Sample No	Mean x [MPL U/ml]	CV [%]
1	8.4	3.7
2	40.1	4.5
3	58.6	5.3

Inter-Assay		
Sample No	Mean x [MPL U/ml]	CV [%]
1	10.3	3.4
2	47.0	3.3
3	79.1	2.5

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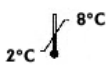
ELISA **E**nzyme **L**inked **I**mmuno**s**orbent **A**ssay



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A – 2351 Wiener Neudorf, Austria, IZ-NÖ Süd, Hondastrasse, Objekt M55
Phone: ++43 (0) 2236 660910-0, Fax: ++43 (0) 2236 660910-30,
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ELISA ENZYME LINKED IMMUNOSORBENT ASSAY

Anti-Phospholipid Screen IgG/IgM

Product Insert

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Anti-Phospholipid Screen IgG/IgM

*Enzyme Linked Immunosorbent Assay for the **quantitative** determination of IgG and IgM autoantibodies against phospholipids in human serum or plasma*

REF

R99449

Content

96 Wells (12 x 8-well antigen coated strips), individual breakaway

For professional in vitro diagnostic use only.

GENERAL INFORMATION

Method	Enzyme Linked Immunosorbent Assay (ELISA)
Shelf life	18 months from date of production
Storage	2-8°C

INTENDED USE

Anti-Phospholipid Screen IgG/IgM is an indirect solid phase immunoassay kit for the quantitative measurement of IgG and IgM class autoantibodies that are directed against β2-glycoprotein mediated anionic phospholipids in human serum or plasma, including Cardiolipin, Phosphatidyl serine, Phosphatidyl inositol, Phosphatidic acid, Phosphatidyl choline, Lyso-phosphatidyl choline and Phosphatidyl ethanolamine.
The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of increased risk of thrombosis in patients with Systemic Lupus Erythematosus (SLE) or similar disorders.
Anti-Phospholipid Screen IgG/IgM is intended for laboratory use only.

DIAGNOSTIC SIGNIFICANCE

The first study on Anti-Phospholipid antibodies began in 1906 when Wasserman introduced a serological test for syphilis. In 1942 it was discovered that the active component was a phospholipid indicated by the name of Cardiolipin. In the 1950s it was observed that a large number of people were tested positive for the syphilis test but did not show any evidence of this disease. At the beginning the phenomenon was classified as a series of false positive syphilis tests, later it emerged that in this group of patients there was a high prevalence of autoimmune disorders including Systemic Lupus Erythematosus (SLE) and Sjogren's syndrome.
The term lupus anticoagulant (LA), used for the first time in 1972, derives from experimental observations in which an increased risk of thrombosis was observed, paradoxically in the presence of some anticoagulant factors; the term LA is not totally correct since the disease occurs more frequently in patients without lupus and is associated with thrombosis rather than abnormal bleeding.
Some years later the role of a cofactor, the β2-glycoprotein I (apolipoprotein H) also called β2GPI, has been investigated and its interactions with anionic phospholipids in human serum/ plasma. This cofactor is a β-globulin with a molecular weight of 50 kDa which is found in plasma at a concentration of about 200 µg/mL. The β2GPI is involved in the regulation of blood coagulation, inhibiting the intrinsic pathway. In vivo β2GPI is associated with negatively charged substances, such as anionic phospholipids, heparin and lipoproteins. The phospholipid binding region is located in its fifth domain.
The acronym "aPL" (anti-phospholipid antibodies) improperly indicates antibodies directed against negatively charged phospholipids, such as Cardiolipin (CL), Phosphatidyl serine (PS), Phosphatidyl inositol (PI) and phosphatidic acid (PA); according to a more correct meaning of the term, anti-phospholipid antibodies are those antibodies directed against the complex between β2GPI and anionic phospholipids capable of binding to the fifth domain of β2GPI. Among these, Cardiolipin is the most commonly used phospholipid as an antigen for determining the aPL with an ELISA. Diagnostic laboratories measure the antibodies directed against the complex between β2GPI and negatively charged phospholipids, such as Phosphatidyl serine (PS), Phosphatidyl inositol (PI) and phosphatidic acid (PA). Some researchers suggest the use of PS instead of Cardiolipin in ELISA assays for a more precise diagnosis. However, these antibodies against phospholipids are less commonly used, even if their use may increase the clinical sensitivity of patient's samples with suspected Anti-Phospholipid Syndrome (APS), but it cannot replace the determination of anti-Cardiolipin autoantibodies.

TEST PRINCIPLE

Anti-Phospholipid Screen IgG/IgM is based on the binding of antibodies in human serum or plasma directed against the antigenic complex between anionic phospholipids (Cardiolipin, Phosphatidyl serine, Phosphatidyl inositol, Phosphatidic acid, Phosphatidyl choline, Lyso-phosphatidyl choline, Phosphatidyl ethanolamine) and β2-Glycoprotein; these complexes are coated on the microplate. Any antibody of IgG class or IgM class in calibrators, controls or prediluted patient samples binds to its respective antigen.
After 60 minutes of incubation, the microplate is washed with wash buffer for removing non-reactive serum components. An anti-human IgG conjugate solution (Enzyme Conjugate IgG) or an anti-human IgM conjugate solution (Enzyme Conjugate IgM) recognizes IgG class or IgM class antibodies, respectively, bound to the immobilized antigens. After a 30-minute incubation any excess enzyme conjugate which is not specifically bound is washed away with the wash buffer. A chromogenic substrate solution containing TMB is then dispensed into the wells. After a 15-minute incubation the color development is stopped by adding the Stop Solution. The color of the solution changes to yellow. The intensity of color is directly proportional to the concentration of IgG or IgM antibodies present in the original sample.
The concentration of IgG or IgM antibodies in the original sample is calculated using a calibration curve.

REAGENT COMPOSITION

Microwell Plate	1 breakable microplate (12 strips x 8 wells) Antigenic phospholipid and β2-Glycoprotein complexes coated on the microwell plate
Calibrators **C_A-C_B-C_C-C_D-C_E**	5 vials, 1.2 mL, **ready to use** Phosphate buffer 0.1 M, NaN_3 <0.1%, human serum
Controls **(positive / negative)**	2 vials, 1.2 mL each, **ready to use** Phosphate buffer 0.1 M, NaN_3 <0.1%, human serum
Sample Diluent	1 vial, 100 mL Phosphate buffer 0.1 M, NaN_3 <0.1%
Enzyme Conjugate IgG	1 vial, 15 mL Anti h-IgG conjugated with horseradish peroxidase (HRP), BSA 0.1%, Proclin <0.0015%
Enzyme Conjugate IgM	1 vial, 15 mL Anti h-IgM conjugated with horseradish peroxidase (HRP), BSA 0.1%, Proclin <0.0015%
Substrate Solution	1 vial, 15 mL H_2O_2-TMB 0.26 g/L (*avoid any skin contact*)
Stop Solution	1 vial, 15 mL Sulfuric acid 0.15 M (*avoid any skin contact*)
Wash Buffer (10x conc.)	1 vial, 50 mL Phosphate buffer 0.2 M, pH 7.4

MATERIAL REQUIRED BUT NOT PROVIDED

- Distilled water
- Automatic dispenser
- Microplate reader (450 nm, 620-630 nm)

REAGENT PREPARATION

- **Preparation of the Calibrators (C_A-C_E) and Controls**

The Calibrators are ready to use and are mixed, so they contain both IgG and IgM antibodies. The Calibrators have the following concentrations:

	C_A	C_B	C_C	C_D	C_E
AU/mL	0	5	10	20	80

The Controls are ready to use.

- **Preparation of the Samples**

All serum and plasma samples have to be prediluted 1:100 with Sample Diluent;
for example, 10 µL of sample may be diluted with 990 µL of Sample Diluent.

- **Preparation of the Wash Buffer**

Dilute the content of the vial of the Wash Buffer (10X) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. It is possible to observe the presence of crystals in the concentrated Wash Buffer; in this case mix at room temperature until the crystals have completely dissolved; for greater accuracy, dilute the entire bottle of the concentrated Wash Buffer to 500 mL. Make sure that the crystals are completely transferred by rinsing of the bottle, then mix until the crystals have completely dissolved.

STORAGE AND STABILITY

Store all reagents between 2-8°C in the dark. The reagents are stable until the expiry date printed on the labels when stored and handled as indicated.
Open the bag of the Microwell Plate only when it is at room temperature and close it immediately after use; once opened, it is stable until the expiry date of the kit.
Once opened, the Calibrators are stable for 6 months at 2-8°C.
The diluted Wash Buffer is stable for 30 days at 2-8°C.

WARNINGS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- All human source material used in the preparation of Calibrators and Controls for this product has been tested and found negative for antibody to HIV 1&2, HBsAg and HCV. However, no test method can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the Calibrator and the Controls should be handled in the same manner as potentially infectious material.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE. However, these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or Proclin 300® as preservatives. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes. Moreover, it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, run large amounts of water through to prevent the formation of azides.
- The Substrate Solution contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to direct sunlight, metals or oxidants. Do not freeze the solution.

PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly**; therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not refill any waste product back into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices**, it is advisable to clean the fluid handling system prior to using the conjugate, ensuring that the procedures of washing, deproteinization and decontamination are effective in preventing contamination of the conjugate; this procedure is highly recommended when the kit is processed using analyzers which are not equipped with disposable tips.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems it is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not take longer than ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the Substrate Solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the Substrate Solution and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Microbiologically contaminated, highly lipemic or haemolysed samples should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

SPECIMEN COLLECTION AND STORAGE

- Either human serum or plasma samples can be used for the test execution.
- Test samples should be clear.
- Contamination from lipemia should be avoided, although it does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C for up to six months.
- Avoid repeated freezing and thawing of serum samples. This may result in a variable loss of autoantibody activity.
- Testing heat-inactivated sera is not recommended.

TEST PROCEDURE

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, store the reagents at 2-8°C immediately; avoid long exposure to room temperature.
- Unused coated microwell strips should be safely stored in the foil pouch with a desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred back into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each Calibrator (C_A-C_E), two for each Control, two for each sample and one for Blank.

Reagent	Calibrator	Sample or Controls	Blank
Calibrator (C_A-C_E)	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate for 60 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells three times with 300 µL of diluted Wash Buffer. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Enzyme Conjugate **IgG or IgM**	**100 µL**	**100 µL**	
Incubate for 30 minutes at room temperature (22-28°C). Remove the contents from each well, wash the wells three times with 300 µL of diluted Wash Buffer. **Washing**: follow the same indications as in the previous point.			
Substrate Solution	**100 µL**	**100 µL**	**100 µL**
Incubate for 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

INTERPRETATION OF RESULTS

For the Anti-Phospholipid Screen IgG/IgM a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable. However, we recommend using a Lin-Log Plot.
First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve that approximates the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

QUALITY CONTROL AND CALIBRATION

- The Anti-Phospholipid Screen IgG/IgM Positive Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
- Since the Positive Control is prediluted, it does not represent a procedural control for the dilution techniques used for the samples.
- Additional suitable control sera can be prepared by aliquoting pooled human serum specimens and storing it at < -20°C.
- In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay should be repeated:
  - The Positive Control is intended to monitor for a significant reagent failure and does not ensure precision at the assay cut-off.
  - This test is only valid if the optical density at 450 nm for the Positive Control as well as for the Calibrator (C_A-C_E) complies with the respective range, which is stated on the Quality Control Certificate enclosed with each test kit.

PERFORMANCE CHARACTERISTICS

- **Precision and reproducibility**

Precision and reproducibility are evaluated by eight repetitions of two positive samples in two different runs with two different lots. Dispensing and washing operations were performed manually by an operator.
The results with standard deviation and coefficient of variation are given below:

	IgG			
Sample	1		2	
	SD	CV	SD	CV
Intra-Assay	1.03	5.9%	1.31	7.4%
Inter-Assay	0.26	9.2%	5.25	11.7%

	IgM			
Sample	1		2	
	SD	CV	SD	CV
Intra-Assay	0.61	7.6%	1.97	5.9%
Inter-Assay	0.15	7.1%	2.98	6.6%

- **Sensitivity**

The clinical sensitivity of Anti-Phospholipid Screen IgG is 92.3%.
The clinical sensitivity of Anti-Phospholipid Screen IgM is 68.8%.

- **Specificity**

The clinical specificity of Anti-Phospholipid Screen IgG is 84.6%.
The clinical specificity of Anti-Phospholipid Screen IgM is >99.9%.

- **Detection limit**

The lowest concentration that can be distinguished from Calibrator A (0 AU/mL) is 0.03 AU/mL for IgG and 0.16 AU/mL for IgM.

TRACEABILITY

Since no international reference preparation for Anti-Phospholipid IgG and IgM autoantibodies is available, the assay system is calibrated in relative arbitrary units.
For calibration of the Anti-Phospholipid Screen IgG/IgM ELISA an internal reference curve has been set up that contains Anti-Phospholipid IgG and IgM antibodies high positive serum.

EXPECTED VALUES

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-Phospholipid Screen IgG/IgM test:

	IgG [GPL AU/mL]	**IgM [MPL AU/mL]**
Normal	< 10	< 10
Elevated	≥ 10	≥ 10

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore, each laboratory should consider the range given by the manufacturer as a general indication and create their own range of expected values based on the indigenous population where the laboratory works.

Positive results should be verified concerning the entire clinical status of the patient. Also, every decision for therapy should be taken individually.
It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-Phospholipid antibodies.

LIMITATIONS

The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and lead to false positive results in this assay.

WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

LITERATURE

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4. Pengo V. et al.: Immunological Specificity and Mechanism of Action of IgG Lupus Anticoagulants. Blood, Vol. 70. N. 1: 69-76, 1987.

CE IVD 8°C 2°C

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Phone: ++43 (0) 2236 660910-0, Fax: ++43 (0) 2236 660910-30
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IZ NOE-Sued, Hondastrasse, Objekt M55, 2351 Wr. Neudorf, Austria
Phone: +43 (0) 2236 660910-0, Fax: +43 (0) 2236 660910-30, e-mail: office@dialab.at

Ferritin

(en) English

REF **K01210**

Content

- 1 Microwell Plate: 96 wells (12 x 8-well antibody coated strips, individual breakaway)
- 5x 1 mL Ferritin Calibrators
- 1x 3 mL Ferritin Calibrator
- 1x 1 mL Ferritin Control
- 1x 21 mL Enzyme Conjugate
- 1x 15 mL Substrate Solution (TMB)
- 1x 15 mL Stop Solution
- 1x 50 mL Wash Buffer (10x conc.)

For professional in vitro diagnostic use only.

INTENDED USE

Ferritin ELISA is a manual in vitro diagnostic device intended for the quantitative determination of ferritin in human serum or plasms. Results are to be used in conjunction with other clinical and laboratory data as an aid in the diagnosis of diseases affecting iron metabolism.

DIAGNOSTIC SIGNIFICANCE

Ferritin is a protein mainly present in the cytoplasm but also found in serum and plasma as an iron-carrier protein. It contains, stores and releases iron in a controlled fashion. One molecule of ferritin is capable of binding between 4000 and 5000 atoms of iron, making ferritin the major iron storage protein for the body[1,2].
Iron is an essential micronutrient as it is required for an adequate erythropoietic function, oxidative mechanism and cellular immune response[1,2,3]. Under physiologic conditions there is a balance between iron absorption, iron transport an iron storage in the human body. When this balance is interrupted, two main conditions can occur: iron deficiency and iron overload[1,2,4]. In humans, ferritin acts as a buffer against iron deficiency and overload.Iron deficiency is the most common and widespread nutritional disorder in the world, affecting 2 billion people and in particular children, women of childbearing age with heavy menstrual flow and miscarriages, and premenopausal women. The WHO estimates that 30% of nonpregnant and 42% of pregnant women are affected by iron deficiency and anemia[5-10].
Since the concentration of ferritin is directly proportional to the total iron stores in the body, serum ferritin concentrations are considered as a common diagnostic tool in the diagnosis of diseases affecting iron metabolism[1].
In addition to ferritin levels additional markers assessed as part of the routine diagnosis of iron deficiency are transferrin saturation and the assessment of haemoglobin levels. All these parameters should also be considered together with other parameters such as the familial history of the patients and the evaluation of clinical symptoms.

TEST PRINCIPLE

The Ferritin ELISA is based on simultaneous binding of human Ferritin to two monoclonal antibodies, one immobilised on the Microwell Plate and the other conjugated with horseradish peroxidase (HRP).
After the incubation, the bound/free separation is performed by a simple solid phase washing. Then, the enzyme HRP in the bound fraction reacts with the Substrate (H_2O_2) and the TMB Substrate and develops a blue colour that changes into yellow when the Stop Solution (H_2SO_4) is added. The colour intensity is proportional to the ferritin concentration of the sample.
Ferritin concentration in the sample is calculated through a calibration curve.

REAGENT COMPOSITION

Component	Description
Microwell Plate	Anti-Ferritin antibody adsorbed on microplate
Ferritin Calibrators	Calibrators containing the following concentrations of Ferritin: C_A C_B C_C C_D C_E C_F ng/mL 0 5 20 100 400 800
Ferritin Control	The Ferritin concentration of the control is lot-specific and is indicated on the Certificate of Analysis
Enzyme Conjugate	Anti-Ferritin antibody conjugated with Horseradish peroxidase (HRP)
Substrate Solution (TMB)	H_2O_2-TMB 0.26 g/L (avoid any skin contact)
Stop Solution	Sulphuric acid 0.15 mol/L (avoid any skin contact)
Wash Buffer	Phosphate buffer 0.2M; pH 7.4 10x concentrated – dilute before use.

MATERIAL REQUIRED BUT NOT PROVIDED

- Distilled water
- Automatic dispenser
- Precision Pipetting Devices
- Microplate reader (450 nm, 620-630 nm)

REAGENT PREPARATION

Preparation of the Wash Buffer:
Dilute the content of the Wash Buffer with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio.

It is possible to observe the presence of crystals within the concentrated wash solution. In this case mix at room temperature until the complete dissolution of crystals. For greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to also transfer crystals completely by rinsing of the bottle. Then mix until crystals are completely dissolved.

Preparation of the Calibrators:
The calibrators are ready to use and are provided at the following concentrations:

	C_A	C_B	C_C	C_D	C_E	C_F
ng/mL	0	5	20	100	400	800

STORAGE AND STABILITY

Store the kit at 2 – 8°C in the dark.

- The kit is stable at 2 – 8°C until the expiry date stated on the external kit label.
- Once opened, the kit is stable at 2 – 8°C for 5 months.
- The diluted wash solution is stable for 30 days at 2 – 8°C.

WARNINGS AND PRECAUTIONS

- This kit is intended for in vitro use by professional persons only. Not for internal or external use in humans or animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Proclin 300® as a preservative. Avoid the contact with skin or mucosa.
  Classification of Proclin 300® under CLP: Skin Sens 1
  Hazard statements:
  H317: May cause allergic skin reaction

  Precautionary statements:
  P261: Avoid breathing dust / fumes / gas / mist / vapours / spray.
  P272: Contaminated work clothing should not be allowed out of the workplace.
  P280: Wear protective gloves / protective clothing / eye protection / face protection.
  P302/352: IF ON SKIN: Wash with plenty of water / soap and water.
  P321: Specific treatment (see instructions on the label)
  P333/313: If skin irritation or rash occurs: get medical attention.
- The Substrate Solution contains an irritant, which may be harmful if inhaled, ingested, or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a dilute sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of the Substrate Solution to direct sunlight, metals, or oxidants. Do not freeze the solution.

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vial labels must be observed. Do not use any kit component beyond their expiry date.

- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems, it is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate.
- Addition of the Substrate Solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the Substrate Solution and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of reagents.
- Samples microbiologically contaminated, highly lipaemic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

In the event of an incident related to the device, report it to the manufacturer and your competent authority as required.

SPECIMEN COLLECTION AND STORAGE

The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin or potassium EDTA) samples.

Sample Storage	Duration
2 – 8°C	72 hours
Freeze / thaw cycles	3 cycles

TEST PROCEDURE

Preparation of Samples:

Ferritin determination should be performed with human serum or plasma.

Procedure:

- Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes. At the end of the assay, immediately store the reagents at 2-8°C. Avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_5), two for the Control, two for each sample, one for Blank.

Pipette:

Reagent	Calibrators	Sample / Control	Blank
Sample / Control		10 µL	
Calibrators C_A – C_F	10 µL		
Enzyme Conjugate	200 µL	200 µL	

Incubate for 1 hour at room temperature (22 – 28°C).
Remove the content form each well. Wash the wells 3 times with 300 µL of diluted Wash Buffer.

Important note: during each manual washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.

Automatic washer: if you use automated equipment, wash the wells at least 5 times.

Pipette:

Substrate Solution	100 µL	100 µL	100 µL
Incubate for 15 minutes at room temperature (22 – 28°C) in the dark			
Stop Solution	100 µL	100 µL	100 µL

Shake the microplate gently.
Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.

INTERPRETATION OF RESULTS

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A cubic spline or 4-parameter logistic (4PL) curve fit, **including Calibrator A is recommended.**

Alternatively, a calibration curve may be prepared on semi-log graph paper by plotting the mean absorbance on the Y-axis against concentration of analyte on the X-axis. Calibrator A should be included in the calibration curve. Read the mean absorbance value of each unknown sample off the curve.

QUALITY CONTROL AND CALIBRATION

Good Laboratory Practice (GLP) requires the use of quality control specimens in each series of assays in order to check the performance of the assay. Controls should be treated as unknown samples, and the results analysed with appropriate statistical methods.

The kit control provided in the kit should be tested as unknown and is intended to assist in assessing the validity of results obtained with each assay plate.

The mean concentration of the control level is documented in the Certificate of Analysis included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple across each plate.

Dialab recommends the users to maintain graphic records of the control values generated with each assay run, including the running means, SDs and %CVs. This information will facilitate the controls trending analysis relating to the performance of current and historical control lots relative to the supplied Quality Control data. The rending will assist in the identification of assays which give control values significantly different form their average range.

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the Certificate of Analysis should be appropriate for assays that are performed manually and with strict adherence to the Test Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[1,2].

PERFORMANCE CHARACTERISTICS

Measuring range:
The measuring range of the assay is 2.84 – 800 ng/mL.
Any value that reads below 2.84 ng/mL should be reported as “< 2.84 ng/mL”. Any value that reads above 800 ng/mL should be reported as “> 800 ng/mL”

Detection Capability:
The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLI EP17-A, “Protocols for Determination of Limits of Quantitation” using 6 blanks and 6 low level samples.

Sensitivity	Concentration
Limit of Blank (LoB)	0.35 ng/mL
Limit of Detection (LoD)	2.00 ng/mL
Limit of Quantification (LoQ)	2.84 ng/mL

Trueness:
Trueness has been demonstrated through method comparison of the Ferritin ELISA to a commercially available assay using native donor samples – refer to subsection “Method Comparison”

Precision:
Precision of the Ferritin ELISA was determined by performing a complex precision study.

Repeatability:
A total of 6 serum samples were assayed in five replicates, once a day for 5 days by 3 operators.
Data from one representative lot is shown below:

Sample	n	Mean Conc. (ng/mL)	Within Laboratory (Reproducibility)	
			SD	CV
1	75	9.21	1.23	13.3%
2	75	68.30	2.47	3.6%

3	75	178.38	9.44	5.3%
4	75	380.97	26.54	7.0%
5	75	467.72	25.58	5.5%
6	75	619.17	51.33	8.3%

Reproducibility:
A total of 6 serum samples were assayed in five replicates, once a day for 5 days by 3 operators.
Results for the combined data from two lots is shown below:

Sample	n	Mean Conc. (ng/mL)	Within Laboratory (Reproducibility)	
			SD	CV
1	150	8.96	1.21	13.5%
2	150	67.85	4.31	6.4%
3	150	177.92	14.14	7.9%
4	150	377.41	32.10	8.5%
5	150	460.17	46.83	10.2%
6	150	614.18	75.43	12.3%

Linearity:
Linearity was evaluated based on CLSI EP-06 “Evaluation of the Linearity of Quantitative Measurement Procedures”. For ferritin concentration by Ferritin ELISA, the measurement procedure shows linearity for the interval from 2.5 to 849 ng/mL within the allowable deviation of linearity (ADL) of ±15%.

Method comparison:
The Ferritin ELISA was compared against a commercially available quantitative automated ferritin assay, following CLSI EP-9A, “Method Comparison and Bias Estimation Using Patient Samples”. A total of 100 samples, selected to represent a wide range of ferritin concentrations (15.65 to 782 ng/mL), were assayed by each method. Passing-Bablok-regression analysis was performed on the comparative data:

n	Slope [95% CI]	Intercept (ng/mL) [95% CI]	Correlation coefficient (r)
100	0.96 (0.92 – 1.01)	0.24 (-5.46 – 3.07)	0.96

Analytical Specificity:
The following substances do not interfere with a bias of > ± 15% in the ferritin determination when the concentrations are below the stated threshold presented in the following table:

Potentially interfering reagent	Threshold concentration
Bilirubin, conjugated	15 mg/dL
Bilirubin, unconjugated	15 mg/dL
Haemoglobin	1000 mg/dL
Total Protein	10 g/dL
Triglyceride	3000 mg/dL

Serum-plasma study:
The Ferritin ELISA matrix comparison study was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma, and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP9-A3 guidelines. A total of 20 samples covering the assay range were evaluated. Passing-Bablok regression analysis was performed on the comparative data:

Sample type	Slope [95% CI]	Intercept (ng/mL) [95% CI]	Correlation coefficient (r)
SST	1.02 (0.99 – 1.25)	-1.02 (-4.98 – 0.28)	1.00
Lithium Heparin	1.04 (0.95 – 1.09)	-1.61 (-3.33 – 0.42)	1.00
Sodium Heparin	1.07 (0.98 – 1.21)	-1.92 (-4.51 - -0.22)	1.00
EDTA	0.97 (0.88 – 1.06)	-1.02 (-3.24 – 0.66)	1.00

Hook effect:
No hook effect was observed with the Ferritin ELISA up to 10,000 ng/mL.

TRACEABILITY

The Dialab Ferritin ELISA has been standardised against internal reference standards (serum matric) which have been value assigned to another commercially available test method.

EXPECTED VALUES

The following ranges were determined using the Ferritin ELISA and are provided for information only. The 95% reference interval for apparently healthy adults were calculated by a non-parametric method following guidance from CLSI C28-A3 “Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory”.

	n	Median (ng/mL)	Reference interval (ng/mL)
Women			
Pre-menopausal	42	33.5	8.4 – 156.9
Post-menopausal	42	44.8	16.8 – 180.8
Men	84	122.5	21.7 – 276.4

LIMITATIONS

- As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient’s clinical presentation and other information available to the physician.
- The performance characteristics of this assay have not been established in a paediatric population.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays[13]. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.

WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

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USED SYMBOLS

Symbol	Description
Cont.	Content
CE	
IVD	
2°C 8°C	

DIALAB Produktion und Vertrieb von chemisch-technischen Produkten und Laborinstrumenten Gesellschaft m.b.H.
IZ NOE-Sued, Hondastrasse, Objekt M55, 2351 Wr. Neudorf, Austria
Phone: +43 (0) 2236 660910-0, Fax: +43 (0) 2236 660910-30, e-mail: office@dialab.at

PSA

(en) English

REF **Z00338**

Content

- 1 Microwell Plate: 96 wells (12 x 8-well antibody coated strips, individual breakaway)
- 5x 0.5 mL Calibrators
- 1x 10 mL Zero Calibrator/ Sample Diluent
- 1x 0.5 mL Control low
- 1x 0.5 mL Control high
- 1x 12 mL Enzyme Conjugate
- 1x 12 mL Substrate Solution (TMB)
- 1x 14 mL Stop Solution
- 1 Certificate of Analysis
- 1 Package Insert

For professional in vitro diagnostic use only.

INTENDED USE

The DIALAB PSA ELISA is an enzyme immunoassay for the quantitative in vitro diagnostic measurement of total prostate specific antigen concentration (t-PSA) in human serum or plasma (EDTA, lithium heparin or citrate plasma). For in vitro diagnostic use only.
The determination of PSA levels is used to estimate the risk of prostate carcinoma in men in conjunction with digital rectal examination (DRE) or to monitor the effectiveness of prostate carcinoma treatment in patients.

DIAGNOSTIC SIGNIFICANCE

Prostate-specific antigen (PSA), also known as gamma-seminoprotein or kallikrein-3 (KLK3), is a glycoprotein enzyme of the kallikrein-related peptidase family. PSA is secreted by the epithelial cells of the prostate gland in very high concentrations to the ejaculate, where it liquefies semen in the seminal coagulum and dissolves cervical mucus, allowing the entry of sperm into the uterus (1,2). PSA circulates in blood in much lower concentrations. The main form of immunoreactive PSA is bound by alpha-1 antichymotrypsin (PSA-ACT), representing approximately 70-80% of the total PSA in the circulation, while the free (uncomplexed) PSA (fPSA; enzymatically inactive) represents 20-30% in serum (3,4). Furthermore, PSA bound to alpha-2 macroglobulin exists in less than 0.1% (undetectable by commercial tests).
In male serum, the normal PSA concentration range is < 4 ng/mL while elevated concentrations of PSA are found in many carcinomas (5). However, increased PSA levels are not only found in patients with prostate cancer, but also in those with a diagnosis of benign prostatic hyperplasia (BPH), acute, subclinical or chronic prostatitis and urinary retention. Analysis of PSA levels in combination with digital rectal examination (DRE) further increase the chance of early detection of prostate cancer. In addition to total PSA, the most useful diagnostic index for distinguishing benign hypertrophy from prostate cancer is the free to total PSA ratio. In order to achieve even better specificity in early detection of prostate cancer, the following indexes may be determined: age-specific PSA, PSA density, acceleration of PSA, and PSA density of the transition zone (6-11).
The determination of PSA serum levels is not only important for the screening of patients for prostate cancer, but also for monitoring patients who have been treated for this disease. Here regular PSA measurements are an important tool to examine the potential and actual effectiveness of surgery or other therapies. An increase of PSA in patients after radical prostatectomy or radiotherapy may allow an earlier discovery of residual or recurrent carcinoma (12-14).
The American Cancer Society recommends to offer PSA blood test and the digital rectal examination annually, beginning at age 50, to men who are at average risk of prostate cancer and who have a life expectancy of at least 10 years. Men at high risk of developing prostate cancer (African Americans or men with a close relative diagnosed with prostate cancer before age 65) should be tested beginning at age 45 (15,16).

TEST PRINCIPLE

This assay is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.
The microtiter wells are coated with a monoclonal (mouse) antibody directed towards a unique antigenic site of the PSA molecule.
During incubation, the PSA molecules in the added sample bind to the immobilized antibody. The added enzyme conjugate, which contains an anti-PSA antibody conjugated to horseradish peroxidase, binds to the PSA forming a sandwich complex.
After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by addition of stop solution, and optical density (OD) of the resulting yellow product is measured. The intensity of colour is proportional to the concentration of the analyte in the sample.

A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

REAGENT COMPOSITION

Each kit contains reagents sufficient for 96 determinations.

Microwell plate	**96 wells** (12 x 8 break-apart strips) Wells coated with anti-PSA antibody (monoclonal)
Zero Calibrator/ Sample Diluent	**1 vial, 10 mL, ready-to-use** Red liquid in a bottle with a transparent cap; contains non-mercury preservative.
Calibrators (C_B-C_C-C_D-C_E-C_F)	**5 vials, 0.5 mL each, ready-to-use** Contains non-mercury preservative. C_B C_C C_D C_E C_F ng/mL 1.56 3.12 6.25 12.5 25.0 *The calibrators (standards) are calibrated against the following reference material: WHO International Standard Prostate Specific Antigen NIBSC code: 96/670.*
Controls (low / high)	**2 vials, 0.5 mL each, ready-to-use** Contains non-mercury preservative. *For control values and ranges, please refer to the Certificate of Analysis.*
Enzyme Conjugate	**1 vial, 12 mL, ready-to-use** Blue liquid in a bottle with a white cap; anti-PSA antibody conjugated to horseradish peroxidase; contains non-mercury preservative.
Substrate Solution	**1 vial, 12 mL, ready-to-use** Black bottle with yellow cap; Tetramethylbenzidine (TMB).
Stop Solution	**1 vial, 14 mL, ready-to-use** Colorless liquid in a white bottle with a red cap; contains 0.5 M H_2SO_4. *Avoid contact with Stop Solution, it may cause skin irritations and burns*.

MATERIAL REQUIRED BUT NOT PROVIDED

- A calibrated microtiter plate reader (450 nm, with reference wavelength at 620 nm to 630 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled water
- Timer
- Graph paper or software for data reduction

REAGENT PREPARATION

Bring all reagents and required number of strips to room temperature (20 °C to 26 °C) prior to use.

STORAGE AND STABILITY

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again.
Opened kits retain activity for 8 weeks if stored as described above.

WARNINGS AND PRECAUTIONS

- This kit is for in vitro diagnostic use only. For professional use only.
- All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
- Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
- The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.

- 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 coloured. 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.
- Allow the reagents to reach room temperature (20 °C to 26 °C) before starting the test. Temperature will affect the optical density readings of the assay. However, values for the patient samples will not be affected.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
- Do not use reagents beyond expiry date as shown on the kit labels.
- All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
- Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
- Avoid contact with Stop Solution containing 0.5 M H2SO4. It may cause skin irritation and burns.
- Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
- TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
- Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
- For information on hazardous substances included in the kit please refer to Safety Data Sheets.
- Safety Data Sheets for this product are available upon request directly from Dialab.
- Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
- Claims submitted due to customer misinterpretation of laboratory results subject to chapter “Expected values” are also invalid. Regardless, in the event of any claim, the manufacturer’s liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.
- Substrate Solution: This reagent contains N-Methyl-2-pyrrolidone, which is classified as below:

Danger

H360D: May damage the unborn child.
P280: Wear protective gloves/ protective clothing/ eye protection/ face protection.

SPECIMEN COLLECTION AND STORAGE

Serum or plasma (EDTA, lithium heparin or citrate plasma) can be used in this assay.
Note: Samples containing sodium azide should not be used in the assay.
In general, it should be avoided to use haemolytic, icteric, or lipaemic specimens. For further information refer to chapter “Interfering Substances”.

Important notes before blood drawing for PSA determination:
As different factors could influence the PSA level in blood, doctors should ensure that the patient has avoided the following conditions before taking the blood sample.
The following conditions may lead to an increase of PSA levels

- Manipulation of the prostate during medical examinations like digital rectal examination (DRE), transrectal prostatic ultrasound, etc.
- Prostatitis
- Biking
- Sexual intercourse (ejaculation)
- Liver dysfunction

The following conditions may lead to a decrease of PSA levels

- Intake of 5-alpha-reductase-inhibitors, antiandrogens, or GnRH analoga

Specimen collection:

- **Serum:**
  Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

- **Plasma:**
  Whole blood should be collected into centrifuge tubes containing anticoagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

Specimen Storage:
Specimens should be capped and may be stored for up to 7 days at 2°C to 8°C prior to assaying. Specimens stored for a longer time (up to 12 months) should be frozen only once at -20°C prior to assay. Thawed sample should be inverted several times prior to testing.

Specimen Dilution:
If in an initial assay a specimen is found to contain more analyte than the highest standard, the specimen can be diluted with Zero Calibrator and re-assayed as described in “Assay Procedure”.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a)	*Dilution 1:10*	*10 µL sample + 90 µL Zero Calibrator (mix thoroughly)*
b)	*Dilution 1:100*	*10 µL dilution a) 1:10 + 90 µL Zero Calibrator (mix thoroughly)*

TEST PROCEDURE

General remarks:

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Optical density is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule, the enzymatic reaction is linearly proportional to time and temperature.

Test procedure:
Each run must include a standard curve.
Note*: It is highly recommended to perform all measurements as duplicates.*

1) Secure the desired number of microtiter wells in the frame holder.
2) Dispense **25 µL** of each **Calibrator, Control** and **sample** with new disposable tips into appropriate wells.
3) Incubate for **5 minutes** at room temperature.
4) Dispense **100 µL** of **Enzyme Conjugate** into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5) Incubate for **60 minutes** at room temperature.
6) Rinse the wells **5 times** with **400 µL** distilled water per well if a plate washer is used.
   -OR-
   Briskly shake out the content of the wells.
   Rinse the wells **5 times** with **300 µL** distilled water per well for manual washing. Strike the wells sharply on absorbent paper to remove residual droplets.
   Important note:
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7) Add **100 µL** of **Substrate Solution** to each well.
8) Incubate for **20 minutes** at room temperature.
9) Stop the enzymatic reaction by adding **100 µL** of **Stop Solution** to each well.
10) Determine the optical density of the solution in each well at **450 nm (reading) and at 620 nm to 630 nm (background subtraction, recommended)** with a microtiter plate reader.
    It is recommended that the wells be read **within 10 minutes** after adding the Stop Solution.

The test must be performed exactly as per the manufacturer’s instructions for use. Moreover, the user must strictly

adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact Dialab.

INTERPRETATION OF RESULTS

1. Calculate the average optical density (OD) values for each set of standards, controls and patient samples.
2. Using graph paper, construct a standard curve by plotting the mean OD obtained from each standard against its concentration with OD value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean OD value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instruction for Use have been calculated automatically using a 4-Parameter curve fit (4-Parameter Rodbard or 4-Parameter Marquardt are the preferred methods). Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as >25 ng/mL. For the calculation of the concentrations this dilution factor has to be taken into account.

Example of a Typical Standard Curve
The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Zero Calibrator (0 ng/mL)	0.05
Calibrator B (1.56 ng/mL)	0.24
Calibrator C (3.12 ng/mL)	0.39
Calibrator D (6.25 ng/mL)	0.74
Calibrator E (12.5 ng/mL)	1.27
Calibrator F (25.0 ng/mL)	2.01

QUALITY CONTROL AND CALIBRATION

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day-to-day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.
In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above-mentioned items without finding any error contact your distributor or Dialab directly.

PERFORMANCE CHARACTERISTICS

- **Assay Dynamic Range**
  The range of the assay is between 0.2 ng/mL – 25.0 ng/mL.

- **Specificity of Antibodies (Cross-Reactivity)**
  The following substances were tested for cross-reactivity of the assay. No interference with the assay was found for:

Substance	Amount added	Cross-reaction
AFP	10 µg/mL	No
CEA	10 µg/mL	No
HCG	10 µg/mL	No
Lactalbumin	10 µg/mL	No

Plasmin-α2-Antiplasmin complex	5 µg/mL	No

- **Sensitivity**

  The Limit of Blank (LoB) is 0.045 ng/mL.
  The Limit of Detection (LoD) is 0.216 ng/mL.
  The Limit of Quantification (LoQ) is 1.172 ng/mL.

- **Reproducibility**
  - **Intra-Assay**
    The within-assay variability was determined by measuring each sample 24 or 32 times per run:

Sample	n	Mean (ng/mL)	CV (%)
1	24	12.5	6.0
2	24	3.4	3.9
3	32	0.8	8.8

  - **Inter-Assay**
    The between-assay variability was determined by measuring each sample with 3 different lots:

Sample	n	n Lots	Mean (ng/mL)	CV (%)
1	75	3	12.3	6.7
2	75	3	3.3	8.0

  - **Inter-Lot**
    The inter-assay (between-lots) variation was determined by measuring each sample 6 times with 3 different kit lots:

Sample	n	Mean (ng/mL)	CV (%)
1	18	13.0	3.4
2	18	3.5	8.8
3	18	12.4	4.1
4	18	3.4	4.1

- **Recovery**
  Samples have been spiked by adding PSA solutions with known concentrations.
  The recovery (%) was calculated by multiplying the ratio of measured and expected values with 100.

Sample	Expected conc. (ng/mL)	Measured conc. (ng/mL)	Recovery (%)
1	6.4	6.4	99.8
2	4.6	4.7	97.6
3	10.1	10.9	92.6

- **Linearity**

  Samples were measured undiluted and in serial dilutions with standard 0. The recovery (%) was calculated by multiplying the ratio of expected and measured values with 100.

	Sample	1	2	3	4
Concentration (ng/mL)		8.84	9.92	14.52	16.69
Average Recovery (%)		106.9	111.2	111.0	103.1
Range of Recovery (%)	**from**	98.2	107.8	108.5	101.1
	to	114.0	113.5	113.0	106.7

- **High Dose Hook Effect**
  Hook effect was not observed in this test up to a concentration of 2000 ng/mL of PSA.

- **Comparison Studies**

A comparison of the DIALAB PSA ELISA (y) and the reference method Access Hybritech PSA (x) using clinical samples gave the following correlation:

$y = 1.108x – 0.054$ $R^2 = 0.952$ $n = 57$

A comparison of the DIALAB PSA ELISA (y) and the reference method DRG:HYBRiD-XL PSA (x) using clinical samples gave the following correlation:

$y = 1.172x + 0.583$ $R^2 = 0.992$ $n = 57$

TRACEABILITY

The calibrators (standards) are calibrated against the following reference material:
WHO International Standard Prostate Specific Antigen NIBSC code: 96/670

EXPECTED VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

In a study conducted with men, using the Dialab PSA Total ELISA the following data were observed:

Population	n	Mean (ng/mL)	Median (ng/mL)	2.5th - 97.5th Percentile (ng/mL)	Range (min. - max.) (ng/mL)
Healthy males	51	1.20	0.86	0 – 4.85	0 – 5.35
Suspicious males (> 4,0 ng/mL)	50	6.56	5.51	2.93 – 14.82	1.33 – 16.94

The generally recommended threshold for follow-up examinations is:

Cut-off value PSA: 4.0 ng/mL

Healthy men generally have a PSA concentration lower than 4.0 ng/mL.
If the PSA concentration is equal or higher than 4.0 ng/mL, follow-up examinations are highly recommended.
This PSA concentration indicates an elevated risk for prostate cancer but might also be caused by benign prostatic hyperplasia (BPH).
Please note that the 4 ng/mL threshold is only a guideline value.
In the literature it is discussed that modifications according to age and ethnological background might be useful e.g. that for younger men the threshold should be lower than for older men.

It is important to keep in mind that some prostate tumors do not cause elevated PSA levels so that PSA measurements should never replace DRE but should only be used in conjunction with digital rectal examination (DRE).

As elevated PSA levels might also be caused by non-cancerous conditions follow-up examinations might try to increase the diagnostic specificity of total PSA values.
In the literature PSA density, PSA velocity and the ratio of free PSA to total PSA (f-PSA/t-PSA) are discussed to improve discrimination between cancerous and non-cancerous conditions and might be used to reduce unnecessary prostate biopsies.
But only a prostate biopsy can finally show if a prostate carcinoma is present or not.

Note: PSA values can only be used to estimate the cancer risk.
They should always be interpreted in conjunction with other clinical findings and should not be used as a sole basis for prostate cancer diagnosis.

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under chapter “TEST PROCEDURE”. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

LIMITATIONS

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handling of samples or modification of this test might influence the results.

- **Interfering Substances**

Haemoglobin* (up to 0.1 mg/mL), Bilirubin (up to 0.2 mg/mL) and Triglyceride (up to 15 mg/mL) have no influence on the assay results.

*at higher concentration hemoglobin results in too high OD values, strongly hemolytic samples should thus be avoided.

- **Drug Interferences**

The following cytostatic drugs were tested. No interference with the assay was found for:

Drug	Concentration tested (µg/mL)
Carboplatin	700.0
Cisplatin	200.0
Calcium Folinate	2.3
Cyclophosphamide	1000.0
5-Fluorouracil	500.0
Doxorubicin HCl	72.0
Dexamethasone	11.0
Diethylstilbestrol	2.0
Flutamide	10.0
Mehtotrexate	50.0

Furthermore, the following hypertension drugs were tested. No interference with the assay was found for:

Drug	Concentration tested (µg/mL)
Simvastatin	0.1
Irbesartan	1.5
Sildenafil Citrate	5.0
Furosemide	200.0

In addition, the following antimicrobial agent was tested.

Compound	Concentration tested (%)
Benzalkonium Chloride	0.5

The antimicrobial agent Benzalkonium Chloride 0.5% shows no interference with the assay.

The following cytostatic drug were tested. No interference with the assay was found for:

Drug	Concentration tested (µg/mL)
Paclitaxel	5.5

Until now no other substances (drugs) are known to us, which have an influence on the measurements of PSA in a sample.

WASTE MANAGEMENT

The disposal of the kit and all used materials/reagents must be performed according to the national regulations. Special information for this product is given in the Safety Data Sheet, section 13.
In case of any damage to the test kit or components, Dialab must be informed in writing, at the latest one week after receiving the kit. Damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed of according to the official regulations.

LITERATURE

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USED SYMBOLS

Symbol	Description
Cont.	Content

CE 0123 IVD 2°C 8°C