



REF 10229
Prothrombin-GM



Instruction Manual

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	Product Ref.	10229
	Product Desc.	Prothrombin-GM
	Manual Rev. No.	005 : 2014-01-15

1 Intended Use

Prothrombin-GM is a solid phase enzyme immunoassay employing native human prothrombin (Factor II) for the quantitative detection of IgG and/or IgM antibodies against prothrombin in human serum.

The assay is a tool in the diagnosis of the antiphospholipid syndrome.

2 Clinical Application and Principle of the Assay

Human Factor II (prothrombin) is a plasme zymogen with a molecular weight of 72 kDa. It assembles with the activated forms of Factor V, Factor X and phospholipid to form a catalytic unit known as the prothrombinase complex. In the presence of calcium ions, the complex cleaves the membrane-associated prothrombin into thrombin, which is then released into the soluble phase.

Antibodies against prothrombin belong to the group of antiphospholipid antibodies specific for negatively charged phospholipids, components of biological membranes, and prothrombin. The presence of anti-phospholipid antibodies has been associated with the clinical features of the so-called antiphospholipid syndrome (APS). Many studies have shown a correlation between these autoantibodies and an enhanced incidence of thrombosis, thrombocytopenia and habitual abortions (as a consequence of placental infarct). The exact mechanism by which pathogenic anti-phospholipid antibodies induce thrombosis is not yet revealed fully.

The occurrence of anti-phospholipid antibodies in patients with SLE and related diseases is typical for a secondary anti-phospholipid syndrome (APS). In contrast, anti-phospholipid antibodies in patients with no other autoimmune diseases characterize the primary anti-phospholipid syndrome (APS).

Antibodies targeting prothrombin alone have recently been described being highly associated with fetal loss in APS patients. Prothrombin antibodies are the the first marker for this serious complication as all other known antiphospholipid antibodies failed to correlate with fetal loss.

Additionally, antibodies targeting a complex of prothrombin and phosphatidylserine have been associated with the second major group of clinical manifestations of the APS, venous and arterial thrombosis. However, they failed to show any correlation with fetal loss.

Therefore, both types of antibodies serve as an important diagnostic tool for differential diagnosis of the heterogenous manifestations of the APS.

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The intensity of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

3 Kit Contents

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 x 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG IgM	1 x 15ml 1 x 15ml	Blue Green	Blue Green	Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H ₂ O ₂)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

4 Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F, for at least 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

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5 Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of the intended use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN₃) as a preservative. NaN₃ may be toxic if ingested or adsorbed by skin or eyes. NaN₃ may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

5.2 General directions for use

In case that the product information, including the labeling, is defective or incorrect please contact the manufacturer or the supplier of the test kit.

Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: We recommend test performance at 30°C/86°F for automated systems.

Never expose components to higher temperature than 37°C/ 98.6°F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.

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6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum samples should be used during the first 8h, respectively stored tightly closed at 2-8°C/35-46°F up to 48h, or frozen at -20°C/-4°F for longer periods

7 Assay Procedure

7.1 Preparations prior to starting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

To avoid mistakes we suggest to mark the cap of the different calibrators.

Samples:

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/35-46°F).

7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

NOTE: If IgG and IgM are determined in parallel, calibrators, controls and samples have to be done for each subclass separately.

For *QUANTITATIVE* interpretation

	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	...	
H	Cal D	NC	...	

CalA: calibrator A

CalB: calibrator B

CalC: calibrator C

CalD: calibrator D

CalE: calibrator E

CalF: calibrator F

PC: positive control



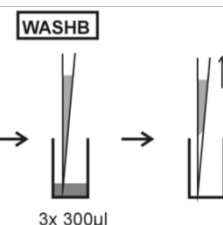
NC: negative control


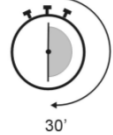
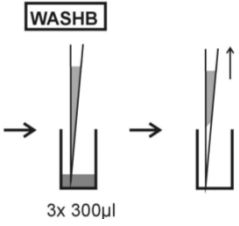

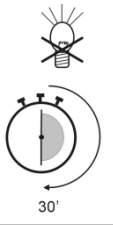
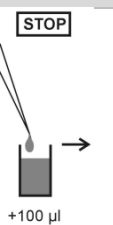

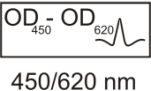
P1: patient 1

P2: patient 2

P3: patient 3

7.3 Test Steps

Step	Description
1.	Ensure preparations from step 7.1 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with quantitative interpretation results desired:
CONTROLS & SAMPLES	
3.	 <p>Pipette into the designated wells as described in chapter 7.2 above, 100 µl of:</p> <p style="padding-left: 40px;">Calibrators (CAL.A to CAL.F)</p> <p>and 100 µl of each of the following:</p> <ul style="list-style-type: none"> • Negative control (NC) and Positive control (PC), and • Patients diluted serum (P1, P2...)
4.	 <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p>
5.	 <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p>

CONJUGATE	
6.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Pipette 100 µl conjugate into each well.</p> </div> </div>
7.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p> </div> </div>
8.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p> </div> </div>
SUBSTRATE	
9.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Pipette 100 µl TMB substrate into each well.</p> </div> </div>
10.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.</p> </div> </div>
STOP	
11.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.</p> </div> </div>
12.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Incubate 5 minutes minimum.</p> </div> </div>
13.	<p>Agitate plate carefully for 5 sec.</p>
14.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.</p> </div> </div>

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8 Quantitative Interpretation

For **quantitative interpretation** establish the standard curve by plotting the **optical density (OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

Example of a standard curve

Do NOT use this example for interpreting patient's result

Calibrators IgG/M	OD 450/620 nm	CV % (Variation)
0 U/ml	0.014	2.9
3 U/ml	0.138	1.0
10 U/ml	0.283	1.5
30 U/ml	0.620	2.3
100 U/ml	1.314	0.9
300 U/ml	2.070	1.2

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.547/0.555	0.551	24.3
P 02	1.638/1.641	1.640	167.4

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

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9 Technical Data

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	90 minutes at 20-32°C/68-89.6°F
Calibration range:	0-300 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C/35-46°F use original vials only.
Number of determinations:	96 tests

10 Performance Data

10.1 Analytical sensitivity

Testing sample buffer 30 times on Prothrombin-GM gave an analytical sensitivity of 1.0 U/ml.

10.2 Specificity and sensitivity

The microplate is coated with **native human prothrombin**. No crossreactivities to other autoantigens have been found. Up to 50 % of pregnant women with APS suffer from fetal loss. Prothrombin identifies 84 % of those.

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	Measured (U/ml)	Expected (U/ml)	Recovery (%)
1	1 / 100	239.0	244.0	98.0
	1 / 200	120.0	122.0	98.4
	1 / 400	59.0	61.0	96.7
	1 / 800	29.4	30.5	96.4
2	1 / 100	172.0	178.0	96.6
	1 / 200	85.0	89.0	95.6
	1 / 400	42.0	44.5	94.3
	1 / 800	20.3	22.3	91.0

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10.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

Intra-assay		
Sample No.	Mean (U/ml)	CV (%)
1	256.0	1.2
2	138.0	3.4
3	64.0	3.3

Inter-assay		
Sample No.	Mean (U/ml)	CV (%)
1	239.0	1.4
2	155.0	3.3
3	58.0	3.2

10.5 Calibration

Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml).

11 Literature




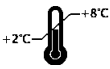

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IVD	<ul style="list-style-type: none"> ◆ Diagnosi in vitro ◆ Pour diagnostic in vitro ◆ In Vitro Diagnostikum ◆ Para uso Diagnóstico in vitro 	<ul style="list-style-type: none"> ◆ For in vitro diagnostic use ◆ Para uso diagnóstico in vitro ◆ In Vitro Διαγνωστικό μέσο ◆ Vücut Dışı Tani Amaçlıdır
REF	<ul style="list-style-type: none"> ◆ Numero di catalogo ◆ Référence Catalogue ◆ Bestellnummer ◆ Número de catálogo 	<ul style="list-style-type: none"> ◆ Catalogue number ◆ Numéro de catálogo ◆ Αριθμός παραγγελίας ◆ Referans Numarası
LOT	<ul style="list-style-type: none"> ◆ Descrizione lotto ◆ Lot ◆ Chargen Bezeichnung ◆ Lote 	<ul style="list-style-type: none"> ◆ Lot ◆ Lote ◆ Χαρακτηρισμός παρτίδας ◆ Lot Numarası
CE	<ul style="list-style-type: none"> ◆ Conformità europea ◆ Déclaration CE de conformité ◆ Europäische Konformität ◆ Declaração CE de Conformidade 	<ul style="list-style-type: none"> ◆ EC Declaration of Conformity ◆ Declaración CE de Conformidad ◆ Ευρωπαϊκή συμμορφία ◆ AT Uygunluk Beyanı
	<ul style="list-style-type: none"> ◆ 96 determinazioni ◆ 96 tests ◆ 96 Bestimmungen ◆ 96 Testes 	<ul style="list-style-type: none"> ◆ 96 tests ◆ 96 pruebas ◆ 96 προσδιορισμοί ◆ 96 Test
	<ul style="list-style-type: none"> ◆ Vedere le istruzioni per l'uso ◆ Voir les instructions d'utilisation ◆ Gebrauchsinformation beachten ◆ Ver as instruções de uso 	<ul style="list-style-type: none"> ◆ See instructions for use ◆ Ver las instrucciones de uso ◆ Αάβετε υπόψη τις οδηγίες χρήσης ◆ Kullanma kilavuzuna bakınız
	<ul style="list-style-type: none"> ◆ Scadenza ◆ Utiliser avant le ◆ Verwendbar bis ◆ Utilizar antes de 	<ul style="list-style-type: none"> ◆ Use by ◆ Utiliz r antes de ◆ Χρήση μέχρι ◆ Son kullanim tarihi
	<ul style="list-style-type: none"> ◆ Conservare a 2-8°C ◆ Conserver à 2-8°C ◆ Lagerung bei 2-8°C ◆ Conservar entre 2-8°C 	<ul style="list-style-type: none"> ◆ Store at 2-8°C (35-46°F) ◆ Conservar a 2-8°C ◆ Φυλάσσεται στους 2-8°C ◆ 2-8 oC'de saklayınız
	<ul style="list-style-type: none"> ◆ Prodotto da ◆ Fabriqué par ◆ Hergestellt von ◆ Fabricado por 	<ul style="list-style-type: none"> ◆ Manufactured b ◆ Fabricado por ◆ Κατασκευάζεται από ◆ Üretici
CON +	<ul style="list-style-type: none"> ◆ Controllo positivo ◆ Contrôle Positif ◆ Positiv Kontrolle ◆ Controllo positivo 	<ul style="list-style-type: none"> ◆ Positive Control ◆ Control Positivo ◆ Θετικός ορός έλέγχου ◆ Pozitif Kontrol
CON -	<ul style="list-style-type: none"> ◆ Controllo Negativo ◆ Contrôle Négatif ◆ Negativ Kontrolle ◆ Controllo negativo 	<ul style="list-style-type: none"> ◆ Negative Control ◆ Control Negativo ◆ Αρνητικός ορός έλέγχου ◆ Negatif Kontrol
CAL	<ul style="list-style-type: none"> ◆ Calibratore ◆ Etalon ◆ Kalibrator ◆ Calibrador 	<ul style="list-style-type: none"> ◆ Calibrator ◆ Calibrador ◆ Αντιδραστήριο βαθμονόμησης ◆ Kalibratör
RC	<ul style="list-style-type: none"> ◆ Recupero ◆ Corrélation ◆ Wiederfindung ◆ Recuperacão 	<ul style="list-style-type: none"> ◆ Recovery ◆ Recuperado ◆ Ανάκτηση ◆ Kurtarma
CONJ	<ul style="list-style-type: none"> ◆ Conjugato ◆ Conjugué ◆ Konjugat ◆ Conjugado 	<ul style="list-style-type: none"> ◆ Conjugate ◆ Conjugado ◆ Σύζευγμα ◆ Konjugat
MP	<ul style="list-style-type: none"> ◆ Micropiastra sensibilizzati ◆ Microplaque sensibilisée ◆ Beschichtete Mikrotiterplatte ◆ Microplaca revestida 	<ul style="list-style-type: none"> ◆ Coated microtiterplate ◆ Microplaca Sensibilizada ◆ Επικαλυμμένη μικροπλάκα ◆ Kaplanmış Mikroplaka
WASHB 50x	<ul style="list-style-type: none"> ◆ Soluzione di lavaggio ◆ Tampon de Lavage ◆ Waschpuffer ◆ Solução de lavagem 	<ul style="list-style-type: none"> ◆ Wash solution ◆ Solución de lavado ◆ Ρυθμιστικό διάλυμα πλύσης ◆ Yıkama solüsyonu
SUB	<ul style="list-style-type: none"> ◆ Tampone substrato ◆ Substrat ◆ Substratpuffer ◆ Substrato 	<ul style="list-style-type: none"> ◆ Substrate buffer ◆ Tampón sustrato ◆ Ρυθμιστικό διάλυμα υποστρώματος ◆ Substrat tamponu
STOP	<ul style="list-style-type: none"> ◆ Reagente bloccante ◆ Solution d'Arrêt ◆ Stopreagenz ◆ Solução de paragem 	<ul style="list-style-type: none"> ◆ Blocking reagent ◆ Solución de parada ◆ Αντιδραστήριο διακοπής αντίδρασης ◆ Stop solüsyonu
SB 5x	<ul style="list-style-type: none"> ◆ Diluente campioni ◆ Tampon Echantillons ◆ Probenpuffer ◆ Diluente de amostra 	<ul style="list-style-type: none"> ◆ Sample Buffer ◆ Tampón Muestras ◆ Ρυθμιστικό διάλυμα δειγμάτων ◆ Numune tamponu