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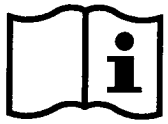

User's Manual

beta2-Glycoprotein I Ab IgG/IgM ELISA

Enzyme immunoassay for the quantitative measurement of IgG and IgM class autoantibodies against beta2-Glycoprotein I in human serum or plasma.

CE

IVD

REF **DE7260**

Σ **96 wells**

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • 24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
info@demeditec.de • www.demeditec.com

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1. INTENDED PURPOSE

Beta2-Glycoprotein I Ag IgG/IgM is an ELISA test system for the quantitative measurement of IgG and IgM class autoantibodies against beta-2-Glycoprotein I in human serum or plasma. This product is intended for professional in vitro diagnostic use only.
Antiphospholipid syndrome (APS, Hughes Syndrome) is a systemic autoimmune disease that causes thromboses, recurrent miscarriage or stillbirths, and stroke. Clinical symptoms are accompanied by specific autoantibodies in the blood, which bind to phospholipids like cardiolipin, or phospholipid-binding proteins like beta-2-glycoprotein I. Autoantibodies against proteins of the coagulation cascade, e.g. prothrombin or annexin V may also be found in patients with APS with otherwise negative phospholipid antibody results. In primary APS autoantibodies against phospholipids appear independently, while in secondary APS phospholipid antibodies are detected in conjunction with other autoimmune diseases, such as lupus erythematosus, rheumatoid arthritis, or Sjögren's syndrome.

2. PRINCIPLE OF THE TEST

Highly purified beta-2-glycoprotein I is bound to microwells.
The determination is based on an indirect enzyme linked immune reaction with the following steps:
Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibody-antigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

3. WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional in vitro diagnostic use only.
- Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
- Stop solution contains acid, classifiaction is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide (NaN_3) 0.09% as preservative. This concentration is classified as non-hazardous.
- Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- Personal precautions, protective equipment and emergency procedures:

Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

4. CONTENTS OF THE KIT

Sufficient for 96 determinations

1. **SORB MT 1 divisible microplate** consisting of 12 modules of 8 wells each. Ready to use.
2. **CAL A – F 6x 1.5 ml Calibrator A-F** (0, 6.3, 12.5, 25, 50, 100 U/ml), containing beta-2-glycoprotein I antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
3. **CONTROL 1 & 2 2x 1.5 ml Control positive (1) and negative (2)**, containing beta-2-glycoprotein I antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.
4. **SAM DIL 5x 20 ml Sample Buffer P,** containing PBS, BSA, detergent, preservative NaN_3 0.09%, yellow, 5x conc.
5. **ENZ CONJ IgG 15 ml Enzyme Conjugate**; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.
6. **ENZ CONJ IgM 15 ml Enzyme Conjugate**; containing anti-human IgM antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.
7. **SUB TMB 15 ml TMB Substrate**; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
8. **STOP SOLN 15 ml Stop solution;** contains acid. Ready to use.
9. **WASH SOLN 50x 20 ml Wash Buffer**, containing Tris, detergent, preservative NaN_3 0.09%; 50 x conc.
10. **1 Instruction for Use**
11. **1 Certificate of Analysis**

5. MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm;
- optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

6. SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum or plasma by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
- Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
- Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
- Testing of heat-inactivated sera is not recommended.

7. STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

8. PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots and products.
- All materials must be at room temperature (20-28°C) prior to use.
- Prepare all reagents and samples. Once started, performe the test without interruption.
- Double determinations may be done. By this means pipetting errors may become obvious.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
- Wash microwells thoroughly and remove the last droplets of wash buffer.
- All incubation steps must be accurately timed.
- Do not re-use microplate wells.

9. PREPARATION OF REAGENTS

Wash Buffer
Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

Sample Buffer
Sample Buffer P: Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples
Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well.

Note: Calibrators / Controls are ready to use and need not be diluted.

10. TEST PROCEDURE

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette 100 µl of calibrators, controls and prediluted patient samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28 °C).
3. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
4. Dispense 100 µl of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
7. Dispense 100 µl of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature
9. Add 100 µl of stop solution to each well of the modules
10. Incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm (reference 600-690nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1	A	P1								
B	B	P2	B	P2								
C	C	P3	C	P3								
D	D	P4	D	P4								
E	E	P5	E	P5								
F	F	P6	F	P6								
G	C+	P7	C+	P7								
H	C-	P8	C-	P8								
	IgG	IgG	IgM	IgM								

P1, ... patient sample A-F calibrators C+, C- controls

11. VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

12. CALCULATION OF RESULTS

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curve by interpolation. Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

13. PERFORMANCE CHARACTERISTICS

Calibration
This assay system is calibrated in relative arbitrary units. Calibration is related to the internationally recognised reference sera from E.N. Harris, Louisville and to IRP 97/656 (IgG) and HCAL (IgG) / EY2C9 (IgM).

Measuring range
The calculation range of this ELISA assay is IgG: 0 - 100 U/ml IgM: 0 - 100 U/ml

Expected values
In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off IgG: 8 U/ml IgM: 8 U/ml

Interpretation of results

	IgG	**IgM**
Negative:	< 5 U/ml	< 5 U/ml
Borderline:	5 - 8 U/ml	5 - 8 U/ml
Positive:	> 8 U/ml	> 8 U/ml

Linearity
Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and the upper / lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observed U/ml	Expected U/ml	O/E %
IgG 1	1:100	100.0	100.0	100
	1:200	49.8	50.0	100
	1:400	25.5	25.0	102
	1:800	13.1	12.5	105
	1:1600	6.9	6.3	110
IgG 2	1:100	80.9	80.9	100
	1:200	42.0	40.5	104
	1:400	21.1	20.2	104
	1:800	10.7	10.1	106
	1:1600	5.6	5.1	110
IgM 1	1:100	97.6	97.6	100
	1:200	49.0	48.8	100
	1:400	23.2	24.4	95
	1:800	13.4	12.2	110
	1:1600	6.4	6.1	105
IgM 2	1:100	70.3	70.3	100
	1:200	33.5	35.2	95
	1:400	18.6	17.6	106
	1:800	10.1	8.8	115
	1:1600	4.9	4.4	111

Limit of detection
Functional sensitivity was determined to be: IgG: 0.5 U/ml IgM: 0.5 U/ml

Reproducibility
Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay IgG		
Sample	**Mean U/ml**	**CV %**
1	8.1	3.3
2	17.8	3.1
3	40.2	3.7

Inter-Assay IgG		
Sample	**Mean U/ml**	**CV %**
1	8.3	6.0
2	17.7	3.4
3	40.1	4.0

Intra-Assay IgM		
Sample	**Mean U/ml**	**CV %**
1	11.5	1.8
2	30.8	2.3
3	66.3	3.1

Inter-Assay IgM		
Sample	**Mean U/ml**	**CV %**
1	11.4	3.0
2	30.7	4.4
3	66.9	2.5

Interfering substances
No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	Pos IgG	%	Pos IgM	%
primary APS	8	6	75.0	4	50.0
secondary APS	65	56	86.2	27	41.5
normal human serum	150	2	1.3	3	2.0

Clinical Diagnosis

IgG	Pos	Neg	
Pos	62	2	
Neg	11	148	
	73	150	223

Sensitivity: 84.9 %
Specificity: 98.7 %
Overall agreement: 94.2 %

Clinical Diagnosis

IgM	Pos	Neg	
Pos	31	3	
Neg	42	147	
	73	150	223

Sensitivity: 42.5 %
Specificity: 98.0 %
Overall agreement: 79.8 %

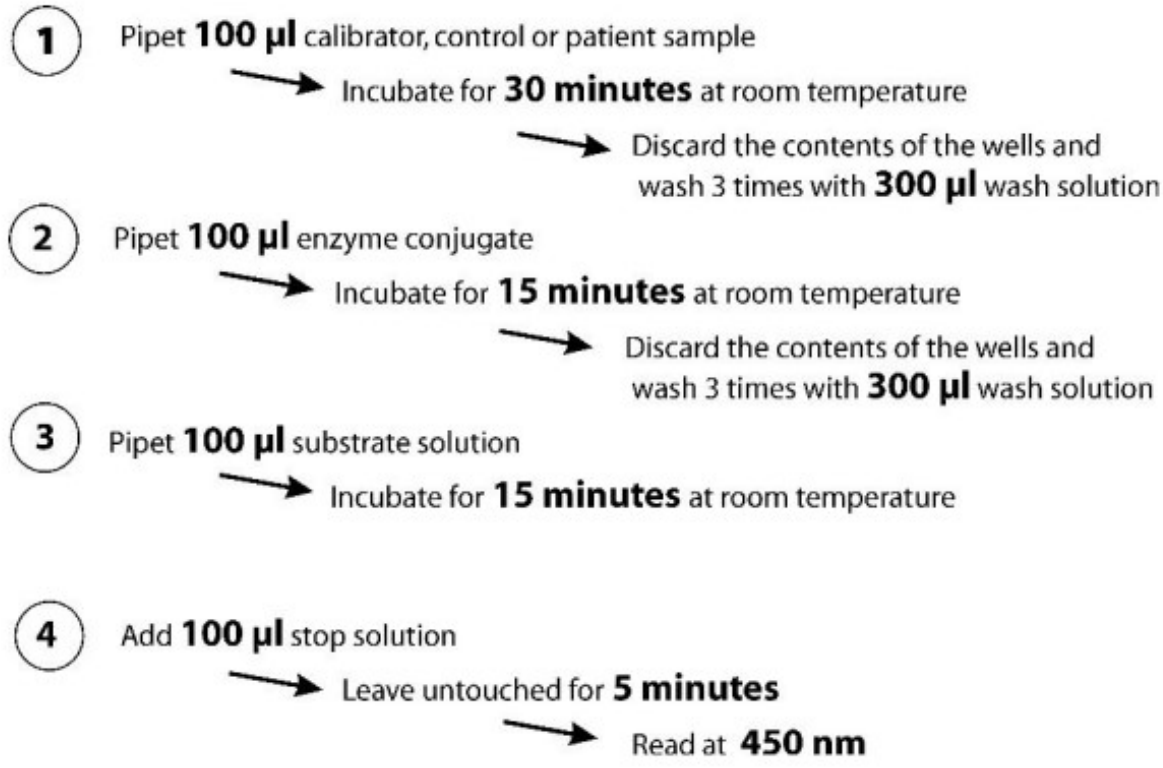
14. LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually. The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

15. REFERENCES

1. Banzato A, Pozzi N, Frasson R, De F, V, Ruffatti A, Bison E et al. Antibodies to Domain I of beta(2)Glycoprotein I are in close relation to patients risk categories in Antiphospholipid Syndrome (APS). Thromb Res 2011; 128(6):583-6.
2. Bertolaccini ML, Amengual O, Atsumi T, Binder WL, de LB, Forastiero R et al. 'Non-criteria' aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, TX, USA, April 2010. Lupus 2011; 20(2):191-205.
3. de Laat B, de Groot PG. Autoantibodies directed against domain I of beta2-glycoprotein I. Curr Rheumatol Rep 2011; 13(1):70-6.
4. de Laat B, Mertens K, de Groot PG. Mechanisms of disease: antiphospholipid antibodies-from clinical association to pathologic mechanism. Nat Clin Pract Rheumatol 2008; 4(4):192-9.
5. de Laat B, Pengo V, Pabinger I, Musial J, Voskuyl AE, Bultink IE et al. The association between circulating antibodies against domain I of beta2- glycoprotein I and thrombosis: an international multicenter study. J Thromb Haemost 2009; 7(11):1767-73.
6. Espinosa G, Cervera R. Antiphospholipid syndrome. Arthritis Res Ther 2008; 10(6):230.
7. Favaloro EJ, Wong RC. Laboratory testing for the antiphospholipid syndrome: making sense of antiphospholipid antibody assays. Clin Chem Lab Med 2011; 49(3):447-61.
8. Fischer MJ, Rauch J, Levine JS. The antiphospholipid syndrome. Arthritis Rheum 2007; 27(1):35-46.
9. Giannakopoulos B, Passam F, Ioannou Y, Krilis SA. How we diagnose the antiphospholipid syndrome. Blood 2009; 113(5):985-94.
10. Greaves M, Cohen H, Machin SJ, Mackie I. Guidelines on the investigation and management of the antiphospholipid syndrome. Br J Haematol 2000; 109 (4):704-15.
11. Hughes GR. Hughes syndrome: antiphospholipid syndrome. J R Coll Physicians Lond 1998; 32(3):260-4.
12. Hughes GR. Hughes Syndrome (the antiphospholipid syndrome): ten clinical lessons. Autoimmun Rev 2008; 7(3):262-6.
13. Hughes GR. Antiphospholipid syndrome, migraine and stroke. Lupus 2010; 19(5):555-6.
14. Hughes GR, Harris NN, Gharavi AE. The anticardiolipin syndrome. J Rheumatol 1986; 13(3):486-9.
15. Koike T, Bohgaki M, Amengual O, Atsumi T. Antiphospholipid antibodies: lessons from the bench. J Autoimmun 2007; 28(2-3):129-33.
16. Lakos G, Favaloro EJ, Harris EN, Meroni PL, Tincani A, Wong RC et al. International consensus guidelines on anticardiolipin and anti-beta2-glycoprotein I testing: report from the 13th International Congress on Antiphospholipid Antibodies. Arthritis Rheum 2012; 64(1):1-10.
17. Mackworth-Young C. Primary antiphospholipid syndrome: a distinct entity? Autoimmun Rev 2006; 5(1):70-5.
18. Miyakis S, Lockshin MD, Atsumi T, Branch DW, Brey RL, Cervera R et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost 2006; 4(2):295-306.
19. Molina JF, Gutierrez-Urena S, Molina J, Uribe O, Richards S, De CC et al. Variability of anticardiolipin antibody isotype distribution in 3 geographic populations of patients with systemic lupus erythematosus. J Rheumatol 1997; 24(2):291-6.
20. Oku K, Atsumi T, Amengual O, Koike T. Antiprothrombin antibody testing: detection and clinical utility. Semin Thromb Hemost 2008; 34(4):335-9.
21. Pengo V, Biasiolo A, Bison E, Chantarangkul V, Tripodi A. Antiphospholipid antibody ELISAs: survey on the performance of clinical laboratories assessed by using lyophilized affinity-purified IgG with anticardiolipin and anti-beta2-Glycoprotein I activity. Thromb Res 2007; 120(1):127-33.
22. Pierangeli SS, de Groot PG, Dlott J, Favaloro E, Harris EN, Lakos G et al. 'Criteria' aPL tests: report of a task force and preconference workshop at the 13th International Congress on Antiphospholipid Antibodies, Galveston, Texas, April 2010. Lupus 2011; 20(2):182-90.
23. Pierangeli SS, Favaloro EJ, Lakos G, Meroni PL, Tincani A, Wong RC et al. Standards and reference materials for the anticardiolipin and anti-beta-2- glycoprotein I assays: a report of recommendations from the APL Task Force at the 13th International Congress on Antiphospholipid Antibodies. Clin Chim Acta 2012; 413(1-2):358-60.
24. Sinico RA, Bollini B, Sabadini E, Di Toma L, Radice A. The use of laboratory tests in diagnosis and monitoring of systemic lupus erythematosus. J Nephrol JID - 9012268 2002; 15 Suppl 6:S20-S27.

25. Tincani A, Andreoli L, Casu C, Cattaneo R, Meroni P. Antiphospholipid antibody profile: implications for the evaluation and management of patients. Lupus 2010; 19(4):432-5.
26. Tincani A, Morozzi G, Afeltra A, Alessandri C, Allegri F, Bistoni O et al. Antiprothrombin antibodies: a comparative analysis of homemade and commercial methods. A collaborative study by the Forum Interdisciplinare per la Ricerca nelle Malattie Autoimmuni (FIRMA). Clin Exp Rheumatol 2007; 25(2):268-74.
27. Wilson WA, Gharavi AE, Koike T, Lockshin MD, Branch DW, Piette JC et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. Arthritis Rheum 1999; 42(7):1309-11.
28. Wong RC, Favaloro EJ, Adelstein S, Baumgart K, Bird R, Brighton TA et al. Consensus guidelines on anti-beta 2 glycoprotein I testing and reporting. Pathology 2008; 40(1):58-63.
29. Wong RC, Gillis D, Adelstein S, Baumgart K, Favaloro EJ, Hendle MJ et al. Consensus guidelines on anti-cardiolipin antibody testing and reporting. Pathology 2004; 36(1):63-8.


1
Pipet 100 µl calibrator, control or patient sample
Incubate for 30 minutes at room temperature
Discard the contents of the wells and wash 3 times with 300 µl wash solution
2
Pipet 100 µl enzyme conjugate
Incubate for 15 minutes at room temperature
Discard the contents of the wells and wash 3 times with 300 µl wash solution
3
Pipet 100 µl substrate solution
Incubate for 15 minutes at room temperature
4
Add 100 µl stop solution
Leave untouched for 5 minutes
Read at 450 nm

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore