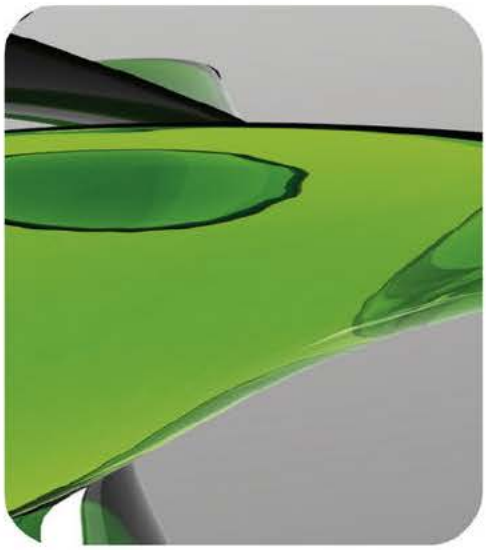
AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS





AESKULISA®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKULISA LKM-1

Ref 3703

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Instruction Manual

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1 Intended Use

AESKULISA LKM-1 is a solid phase enzyme immunoassay employing human recombinant cytochrome p450 IID6 for the quantitative and qualitative detection of antibodies against liver-kidney microsomes (LKM) in human serum.

The assay is a tool for the diagnosis of autoimmune hepatitis (AIH).

2 Clinical Application and Principle of the Assay

Autoimmune hepatitis (AIH) is a chronic progressive liver disease of unknown origin that responds well to immunosuppressive therapy, but has a poor prognosis if untreated. Early and accurate diagnosis is therefore of great importance. AIH is characterized by histological features of periportal hepatitis in the absense of viral markers, by hypergammaglobulinemia and, in the majority of patients, by the presence of autoantibodies in serum. Anti-nuclear antibodies (ANA), smooth muscle antibodies (SMA), anti-liver kidney microsomal antibodies (LKM) and antibodies against soluble liver antigen (SLA) are marker autoantibodies for AIH. 52% of AIH patients are positive for ANA and/or SMA, 20% for SLA and 3% for LKM-1. These antibodies are of diagnostic value for AIH but the only autoantibodies highly specific for AIH are SLA. ANA/SMA also occur in 10-15% of patients with viral hepatitis and other immune-mediated diseases. LKM-1 are also associated with hepatitis C.

Three types of LKM antibodies can be distinguished according to the target antigens. LKM-1 antibodies are directed against cytochrome p450 IID6, a 50 kDa cytoplasmic protein found in hepatocytes and renal proximal tubular cells. LKM-2 antibodies are associated with ticrynafen (tienilic acid) -induced hepatitis. The target antigen is cytochrome p450 IIC9, a cytochrome p450 isoenzyme that catalyzes the metabolic oxidation of the drug. LKM-3 antibodies are associated with chronic hepatitis D. The target antigen is UDP-1 glucoronosyl transferase.

LKM-1 associated AIH predominantly occurs in girls between 2 and 14 years of age, thus determination of LKM-1 is very important in pediatrics.

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)
Cut-off Calibrator	1 x 1.5ml	Blue	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each cal brator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15ml	Blue	Blue	Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethy benzidine and hydrogen peroxide (TMB/H_2O_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 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.

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 (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. NaN_3 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.

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:

For *QUANTITATIVE* interpretation

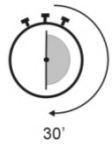
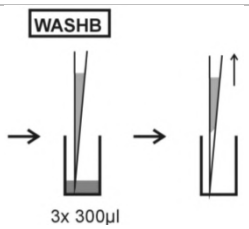
	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	…	
H	Cal D	NC	…	

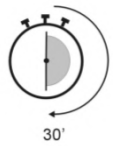
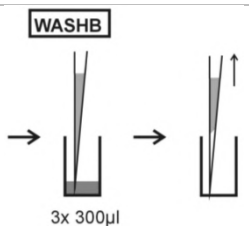
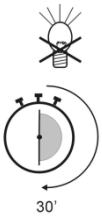
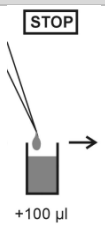
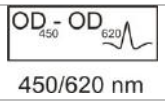
For *QUALITATIVE* interpretation

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

CalA: calibrator A
CalB: calibrator B
CalC: calibrator C
CalD: calibrator D
CalE: calibrator E
CalF: calibrator F
PC: positive control
NC: negative control
CC: cut-off calibrator
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/ qualitative interpretation results desired:
	CONTROLS & SAMPLES
3.	 Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either: a. Calibrators (CAL.A to CAL.F) for *QUANTITATIVE* or b. Cut-off Calibrator (CC) for *QUALITATIVE* interp. and 100 µl of each of the following: Negative control (NC) and Positive control (PC), and Patients diluted serum (P1, P2...)
4.	 Incubate for 30 minutes at 20-32°C/68-89.6°F.
5.	 Wash 3x with 300 µl washing buffer (diluted 1:50).

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

8 Quantitative and Qualitative 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	OD 450/620 nm	CV % (Variation)
0 U/ml	0.046	2.4
3 U/ml	0.171	2.6
10 U/ml	0.372	1.0
30 U/ml	0.698	3.8
100 U/ml	1.456	0.4
300 U/ml	2.396	2.0

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.533/0.569	0.551	19.8
P 02	1.156/1.196	1.176	68.7

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.

For **qualitative interpretation** read the optical density of the cut-off calibrator and the patient samples. Compare patient´s OD with the OD of the cut-off calibrator. For qualitative interpretation we recommend to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

Negative:		**OD patient**	**<**	**0.8 x OD cut-off**		
Equivocal:	**0.8 x**	**OD cut-off**	**≤**	**OD patient**	**≤**	**1.2 x OD cut-off**
Positive:		**OD patient**	**>**	**1.2 x OD cut-off**		

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 AESKULISA LKM-1 gave an analytical sensitivity of 1.0 U/ml.

10.2 Specificity and sensitivity

The microplate is coated with recombinant human cytochrome p450 IID6. No crossreactivities to other autoantigens have been found. Anti-LKM-1 antibodies show a diagnostic specificity of >99% for autoimmune hepatitis type 2. The diagnostic sensitivity of anti-LKM-1 antibodies for autoimmune hepatitis type 2 is 84%.

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	78.9	80.0	98.6
	1 / 200	39.8	40.0	99.5
	1 / 400	18.9	20.0	94.5
	1 / 800	9.6	10.0	96.0
2	1 / 100	34.2	33.0	103.6
	1 / 200	17.2	16.5	104.2
	1 / 400	8.1	8.3	97.6
	1 / 800	4.0	4.2	95.2

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	210.0	1.6
2	77.5	2.8
3	18.4	3.6

Inter-assay		
Sample No.	**Mean (U/ml)**	**CV (%)**
1	207.0	4.2
2	73.8	2.3
3	17.6	1.5

10.5 Calibration

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

11 Literature

Krawitt EL (1996). Autoimmune Hepatitis. N Engl J Med 334: 897-903.

Meyer zum Büschenfelde KH, Lohse AW (1995). Autoimmune Hepatitis. N Engl J Med 333: 1004-1005.

Alvarez F, Berg PA, Bianchi et al. (1999). International Autoimmune Hepatitis Group Report: a review of criteria for diagnosis of auto immune hepatitis. J Hepatol 31: 929-938.

Manns MP et al. (1991). LKM-1 autoantibodies recognize a short linear sequence in P450 IID6, a cytochrome P-450 monooxygenase. J Clin Invest 88: 1370-1378.

Homberg JC, Andre C, Abuaf A (1984). A new anti-liver-kidney microsome antiboda (anti-LKM-2) in tienilic acid-induced hepatitis. Clin Exp Immunol 55: 561-570.

Philipp T, Durazzo M, Trautwein C, Alex B, Straub P, Lamb JG, Johnson EF, Tukey RH, Manns MP (1994). Recognition of uridine diphosphate glucuronosyl transferases by LKM-3 antibodies in chronic hepatitis D. Lancet 344:578-81

IVD	- Diagnosi in vitro	- For in vitro diagnostic use
	- Pour diagnostic in vitro	- Para uso diagnóstico in vitro
	- In Vitro Diagnostikum	- In Vitro Διαγλφζ ηθό κέζο
	- Para uso Diagnóstico in vitro	
REF	¨ Numero d'ordine	¨ Cataloge number
	¨ Référence Catalogue	¨ Numéro de catálogo
	¨ Bestellnummer	¨ Αριζκός παραγγει ίας
	¨ Número de catálogo	
LOT	¨ Descrizione lotto	¨ Lot
	¨ Lot	¨ Lote
	¨ Chargen Bezeichnung	¨ Χαραθηε ριζκός παρηίδας
	¨ Lote	
CE	¨ Conformità europea	¨ EC Declaration of Conformity
	¨ Déclaration CE de Conformité	¨ Declaración CE de Conformidad
	¨ Europäische Konformität	¨ Εσρφπαχθή ζσκθφλία
	¨ Déclaracão CE de Conformidade	
Σ 96	¨ 96 determinazioni	¨ 96 tests
	¨ 96 tests	¨ 96 pruebas
	¨ 96 Bestimmungen	¨ 96 προζδιφριζκοί
	¨ 96 Testes	
i	¨ Rispettare le istruzioni per l'uso	¨ See instructions for use
	¨ Voir les instructions d'utilisation	¨ Ver las instrucciones de uso
	¨ Gebrauchsanweisung beachten	¨ Λάβεηε σπόυ ε ηπ οδεγίες τρήζ ε ς
	¨ Ver as instrucões de uso	
	¨ Da utilizzarsi entro	¨ Use by
	¨ Utilise avant le	¨ Utilizar antes de
	¨ Verwendbar bis	¨ Χρήζ ε κέτρη
	¨ Utilizar antes de	
+2°C +8°C	¨ Conservare a 2-8°C	¨ Store at 2-8°C (35-46°F)
	¨ Conserver à 2-8°C	¨ Conservar a 2-8°C
	¨ Lagerung bei 2-8°C	¨ Φσιάζ ζεηαηζ ρσς 2-8°C
	¨ Conservar entre 2-8°C	
	¨ Prodotto da	¨ Manufactured by
	¨ Fabriqué par	¨ Fabricado por
	¨ Hergestellt von	¨ Καηαζ θεσάδεηαηαπό
	¨ Fabricado por	
CO-CAL	¨ Calibratore cut-off	¨ Cut off Calibrator
	¨ Etalon Seuil	¨ Calibrador de cut-off
	¨ Grenzwert Kalibrator	¨ Οριφθός ορός Αληθραζ ήριφ βαζ κολόκε ζ ε ς
	¨ Calibrador de cut-off	
CON +	¨ Controllo positivo	¨ Positive Control
	¨ Contrôle Positif	¨ Control Positivo
	¨ Positiv Kontrolle	¨ Θεηθός ορός ειέ γτοσ
	¨ Controlo positivo	
CON −	¨ Controllo negativo	¨ Negative Control
	¨ Contrôle Négatif	¨ Control Negativo
	¨ Negativ Kontrolle	¨ Αρλεηθός ορός ειέ γτοσ
	¨ Controlo negativo	
CAL	¨ Calibratore	¨ Calibrator
	¨ Etalon	¨ Calibrador
	¨ Kalibrator	¨ Αληθραζ ήριφ βαζ κολόκε ζ ε ς
	¨ Calibrador	
RC	¨ Recupero	¨ Recovery
	¨ Corrélation	¨ Recuperado
	¨ Wiederfindung	¨ Αλάθηε ζ ε
	¨ Recuperacão	
CONJ	¨ Coniugato	¨ Conjugate
	¨ Conjugé	¨ Conjugado
	¨ Konjugat	¨ Σύδεσγκα
	¨ Conjugado	
MP	¨ Micropiastra rivestita	¨ Coated microtiter plate
	¨ Microplaque sensibilisée	¨ Microplaca sensibilizada
	¨ Beschichtete Mikrotiterplatte	¨ Επηφαισ κκέλε κηθροπιάθα
	¨ Microplaca revestida	
WASHB 50x	¨ Tampone di lavaggio	¨ Wash buffer
	¨ Tampon de Lavage	¨ Solución de lavado
	¨ Waschpuffer	¨ Ροζ κηζ ηθό δηφι σκα πιύ ζ ε ς
	¨ Solucão de lavagem	
SUB	¨ Tampone substrato	¨ Substrate buffer
	¨ Substrat	¨ Tampón sustrato
	¨ Substratpuffer	¨ Ροζ κηζ ηθό δηφι σκα σποζ ηρώκαηρος
	¨ Substrato	
STOP	¨ Reagente bloccante	¨ Stop solution
	¨ Solution d'Arrêt	¨ Solución de parada
	¨ Stopreagenz	¨ Αληθραζ ήριφ δηφθοπής αληθραζ ε ς
	¨ Solucão de paragem	
SB 5x	¨ Tampone campione	¨ Sample buffer
	¨ Tampon Echantillons	¨ Tampón Muestras
	¨ Probenpuffer	¨ Ροζ κηζ ηθό δηφι σκα δεηγκάηρλ
	¨ Diluente de amostra	

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512_3

ORG 512 Anti-Scl-70

INTENDED PURPOSE

Anti-Scl-70 is an ELISA test system for the quantitative measurement of IgG class autoantibodies against Scl-70 in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Antibodies against Scl-70 (DNA topoisomerase I) are an accepted marker for progressive systemic scleroderma. They contribute to the differential diagnosis of scleroderma. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
512_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified Scl-70 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 stopps 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.

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 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.

CONTENTS OF THE KIT

ORG 512	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Color code on module
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 12.5 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 25 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 100 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 U/ml, containing Scl-70 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing Scl-70 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.
CONTROL −	1x 1.5 ml	Control negative, containing Scl-70 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.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

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.

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.

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 dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. 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.

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.

PREPARATION OF REAGENTS

WASH

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.

DILUENT

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.

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.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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

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.

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.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA.

Measuring range

The calculation range of this ELISA assay is 0 - 200 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 25 U/ml

Interpretation of results

Negative:	< 15 U/ml
Borderline:	15 - 25 U/ml
Positive:	> 25 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 [%]
1	1:100	146.9	146.9	100
	1:200	76.3	73.5	104
	1:400	38.1	36.7	104
	1:800	18.8	18.4	102
2	1:100	122.3	122.3	100
	1:200	60.4	61.2	99
	1:400	29.6	30.6	97
	1:800	14.8	15.3	97

Limit of detection

Functional sensitivity was determined to be: 1 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		
Sample	Mean U/ml	CV %
1	45.7	4.0
2	90.4	3.2
3	184.1	3.4

Inter-Assay		
Sample	Mean U/ml	CV %
1	41.1	2.8
2	89.9	2.8
3	157.4	2.3

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, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Scleroderma	25	19	76.0
Rheumatoid arthritis	20	0	0.0
Normal human sera	80	1	1.3

		Clinical Diagnosis POS	NEG	
ORG 512	POS	19	1	
	NEG	6	99	
		25	100	125

Sensitivity: 76.0 %
Specificity: 99.0 %
Overall agreement: 94.4 %

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 establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. Ann Rheum Dis 2003; 62(6):556-560.
2. Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. Lupus 2010; 19(8):906-912.
3. Brouwer R, Hengstman GJ, Vree EW, Ehrfeld H, Bozic B, Ghirardello A et al. Autoantibody profiles in the sera of European patients with myositis. Ann Rheum Dis 2001; 60(2):116-123.
4. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. J Allergy Clin Immunol 2010; 125(2 Suppl 2):S238-S247.
5. Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. Autoimmun Rev 2011; 10(3):150-154.
6. Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of Systemic Lupus Erythematosus in northern Sweden. Arthritis Research & Therapy 2011; 13(1):R30.
7. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. Ann Rheum Dis JID - 0372355 2004; 63 (4):386-394.
8. Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V et al. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. Lupus 2011; 20(3):250-255.
9. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology (Oxford) 2007; 46(7):1052-1056.
10. Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. Immunol Rev 2010; 233(1):126-145.
11. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. Diagn Pathol 2009; 4:1.
12. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis 2010; 69:1420 -1422.
13. Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. Lupus 2004; 13(11):829 -837.
14. Poole BD, Schneider RI, Guthridge JM, Velte CA, Reichlin M, Harley JB et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. Arthritis Rheum 2009; 60(3):848-859.
15. Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent assays in patients with systemic lupus erythematosus and other autoimmune systemic diseases. Ann N Y Acad Sci 2007; 1109:275-286.
16. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. Lupus JID - 9204265 2004; 13(5):290-297.
17. Simon JA, Cabiedes J, Ortiz E, Alcocer-Varela J, Sanchez-Guerrero J. Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker. Rheumatology (Oxford) 2004; 43(2):220-224.
18. Sinclair D, Saas M, Williams D, Hart M, Goswami R. Can an ELISA replace immunofluorescence for the detection of anti-nuclear antibodies?--The routine use of anti-nuclear antibody screening ELISAs. Clin Lab 2007; 53(3-4):183-191.
19. Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. Am J Clin Pathol 2002; 117(2):316-324.
20. Maidhof W., Hilias O. Lupus: an pverview of the disease and management options. P T 2012; 37(4):240-9.
21. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. Arthritis Care Res (Hoboken) 2012; 64(6):797-808.

Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 512_IFU_EN_QM113139_2013-12-16_1.2* Reason for revision: *Introduction electronic IFU on homepage*

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**

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515_3

ORG 515 Anti-Cardiolipin IgG/IgM

INTENDED PURPOSE

Anti-Cardiolipin IgG/IgM is an ELISA test system for the quantitative measurement of IgG and IgM class autoantibodies against cardiolipin 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.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
515_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE G	Enzyme Conjugate
CONJUGATE M	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified cardiolipin is coated on microwells saturated with beta-2-glycoprotein I.
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 stopps 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.

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 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.

CONTENTS OF THE KIT

ORG 515	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***CLP***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 GPL-U/ml / 0 MPL-U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 7.5 GPL-U/ml / 5 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent,NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 15 GPL-U/ml / 10 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 30 GPL-U/ml / 20 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 60 GPL-U/ml / 40 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 120 GPL-U/ml / 80 MPL-U/ml, containing Cardiolipin antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing cardiolipin 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.
CONTROL −	1x 1.5 ml	Control negative, containing cardiolipin 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.
DILUENT	20 ml	Sample Buffer P; containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate 5x.
CONJUGATE G	15 ml	Enzyme Conjugate IgG; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
CONJUGATE M	15 ml	Enzyme Conjugate IgM; containing anti-human IgM antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate, containing 3,3', 5,5'- Tetramethylbenzidin. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
i	1	Certificate of Analysis

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.

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.

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 dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production.
  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.

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.

PREPARATION OF REAGENTS

WASH

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.

DILUENT

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.

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.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µl** of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µl** of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   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

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.

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.

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognised reference sera from E.N. Harris, Louisville and the specific reference material IRP 97/656 (IgG) and HCAL (IgG) / EY2C9 (IgM).

Measuring range

The calculation range of this ELISA assay is IgG: 0 - 120 GPL-U/ml IgM: 0 - 80 MPL-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: 10 GPL-U/ml IgM: 7 MPL-U/ml

Interpretation of results

Negative:	IgG < 10 GPL-U/ml	IgM < 7 MPL-U/ml
Positive:	≥ 10 GPL-U/ml	≥ 7 MPL-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 Factor	Observed GPL/MPL-U/ml	Expected GPL/MPL-U/ml	O/E [%]
IgG 1	1	73.0	73.0	100
	2	37.1	36.5	102
	4	19.6	18.3	107
	8	10.9	9.1	120
IgG 2	1	80.5	80.5	100
	2	42.0	40.3	104
	4	22.2	20.1	111
	8	12.1	10.1	120
IgG 3	1	66.2	64.4	103
	2	34.5	32.2	107
	4	16.2	16.1	101
	8	8.1	8.1	101
IgM 1	1	70.9	70.9	100
	2	34.1	35.5	96
	4	18.2	17.7	103
	8	10.1	8.9	114
IgM 2	1	114.0	114.0	100
	2	50.6	57.0	89
	4	27.3	28.5	96
	8	14.8	14.3	104
IgM 3	1	48.2	48.2	100
	2	24.7	24.1	102
	4	12.7	12.1	105
	8	7.1	6.0	118

Limit of detection

Functional sensitivity was determined to be: IgG: 1 GPL-U/ml IgM: 0.5 MPL-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 GPL-U/ml	CV %
1	10.9	5.5
2	20.5	5.4
3	73.0	5.4

Inter-Assay IgG		
Sample	Mean GPL-U/ml	CV %
1	11.8	5.3
2	21.1	3.7
3	70.5	6.3

Intra-Assay IgM		
Sample	Mean MPL-U/ml	CV %
1	12.8	3.7
2	30.7	4.1
3	65.2	3.8

Inter-Assay IgM		
Sample	Mean MPL-U/ml	CV %
1	12.2	3.5
2	31.4	3.5
3	64.9	4.2

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, Heparine). 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	57	87.7	26	40.0
Normal human serum	150	6	4.0	3	2.0

		Clinical Diagnosis POS	NEG	
ORG 515 IgG	POS	63	6	
	NEG	10	144	
		73	150	223
Sensitivity:	86.3 %			
Specificity:	96.0 %			
Overall agreement:	92.8 %			

		Clinical Diagnosis Pos	Neg	
ORG 515 IgM	Pos	30	3	
	Neg	43	147	
		73	150	223
Sensitivity:	41.1 %			
Specificity:	98.0 %			
Overall agreement:	79.4 %			

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 establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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.

Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 515_IFU_EN_QM113142_2016-04-18_2* Reason for revision: *Introduction electronic IFU on homepage*

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**

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518_3

ORG 518 Anti-PR3 (cANCA)

INTENDED PURPOSE

Anti-PR3 is an ELISA test system for the quantitative measurement of IgG class autoantibodies against proteinase 3 (PR3) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Anti-neutrophil cytoplasmic antibodies (ANCA) are diagnostic markers for ANCA-associated vasculitides. Anti-PR3 characterises granulomatosis with polyangiitis (GPA, formerly: Wegener's granulomatosis). The test supports the differential diagnosis of vasculitis when used in combination with other laboratory and clinical findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
518_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified Proteinase 3 (PR3) 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 stopps 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.

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 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.

CONTENTS OF THE KIT

ORG 518	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***PR3***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 5 U/ml, containing PR3 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 10 U/ml, containing PR3 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 20 U/ml, containing PR3 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 40 U/ml, containing PR3 antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 100 U/ml, containing PR3 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing PR3 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.
CONTROL −	1x 1.5 ml	Control negative, containing PR3 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.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

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.

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.

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 dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. 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.

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.

PREPARATION OF REAGENTS

WASH

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.

DILUENT

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.

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.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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

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.

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.

PERFORMANCE CHARACTERISTICS

Calibration

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

The calculation range of this ELISA assay is 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 5 U/ml

Interpretation of results

Negative: < 5 U/ml
Positive: ≥ 5 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 [%]
1	1:100	78.9	78.9	100
.	1:200	39.8	39.5	101
.	1:400	20.6	19.7	105
.	1:800	10.6	9.9	107
.	1:1600	5.3	4.9	108
2	1:100	77.5	77.5	100
.	1:200	37.4	38.8	96
.	1:400	19.1	19.4	98
.	1:800	9.7	9.7	100
.	1:1600	5.0	4.8	104

Limit of detection

Functional sensitivity was determined to be: 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		
Sample	Mean U/ml	CV %
1	10.9	4.7
2	24.6	2.8
3	58.5	2.8

Inter-Assay		
Sample	Mean U/ml	CV %
1	10.4	6.2
2	23.4	8.8
3	60.7	3.9

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, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Morbus Wegener (c-ANCA pos,	61	52	85.2
vasculitis (pANCA-positive)	20	0	0.0
infammatory/Non-inflammatory	150	3	2.0
Normal human sera	80	0	0.0

		Immunological Diagnosis POS	NEG	
ORG 518	POS	52	3	
	NEG	9	247	
		61	250	311

Sensitivity: 85.2 %
Specificity: 98.8 %
Overall agreement: 96.1 %

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 establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. Jennette, J. C. and Falk, R.J . Antineutrophil Cytoplasmic Autoantibodies and Associated Diseases: a Review. Am. J. Kidney Dis. 1990, Vol. XV, No. 6: 517 - 529.
2. Gross, W. L. et al. Antineutrophil Cytoplasmic Autoantibody-Associated Diseases: A Rheumatologist's Perspective. Am. J. Kidney Dis. 1991, Vol. XVIII, No. 2: 175 - 179.
3. Wieslander, J. How are Antineutrophil Cytoplasmic Autoantibodies Detected ? Am. J. Kidney Dis. 1991, Vol. XVIII, No. 2: 154 - 158.
4. Lesavre, P. Antineutrophil cytoplasmic antibodies antigen specificity. Am. J. Kidney Dis. 1991,Vol. XVIII,No. 2: 159 - 163.
5. Hagen, E. C. et al. Antineutrophil cytoplasmic autoantibodies: a review of the antigens involved, the assays, and the clinical and possible pathogenic consequences. Blood 1993, Vol.81: 1996 - 2000.
6. Gross, W .L. et al. Immunodiagnostische und immunopathogenetische Bedeutung von Anti-Neutrophilen-Cytoplasma-Antikörpern. Deutsche Medizinische Wochenschrift 1993, Vol. 118: 191 - 199.

Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 518_IFU_EN_QM113147_2013-12-16_1.2* Reason for revision: *Introduction electronic IFU on homepage*

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**

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519_3

ORG 519 Anti-MPO (pANCA)

INTENDED PURPOSE

Anti-MPO is an ELISA test system for the quantitative measurement of IgG class autoantibodies against myeloperoxidase (MPO) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Anti-neutrophil cytoplasmic antibodies (ANCA) are diagnostic markers for ANCA-associated vasculitides. Anti-MPO differentiates microscopic polyangiitis (MPA) and eosinophilic granulomatosis with polyangiitis (EGPA) The test supports differential diagnosis of vasculitis, when used in conjunction with other clinical and laboratory findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
Σ 96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
519_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified myeloperoxidase (MPO) 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 stopps 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.

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 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.

CONTENTS OF THE KIT

ORG 519	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***MPO***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 5 U/ml, containing MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 10 U/ml, containing MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 20 U/ml, containing MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 40 U/ml, containing MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 100 U/ml, containing MPO antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing MPO 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.
CONTROL −	1x 1.5 ml	Control negative, containing MPO 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.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

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.

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.

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 dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production.
  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.

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.

PREPARATION OF REAGENTS

WASH

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.

DILUENT

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.

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.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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

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.

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.

PERFORMANCE CHARACTERISTICS

Calibration

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

The calculation range of this ELISA assay is 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 5 U/ml

Interpretation of results

Negative: < 5 U/ml
Positive: ≥ 5 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 [%]
1	1:100	87.3	87.3	100
.	1:200	44.1	43.7	101
.	1:400	21.5	21.8	99
.	1:800	9.7	10.9	89
.	1:1600	5.0	5.5	91
2	1:100	79.9	79.9	100
.	1:200	39.3	40.0	98
.	1:400	19.0	20.0	95
.	1:800	8.5	10.0	85
.	1:1600	4.3	5.0	86

Limit of detection

Functional sensitivity was determined to be: 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		
Sample	Mean U/ml	CV %
1	7.5	6.4
2	30.2	4.1
3	59.9	3.1

Inter-Assay		
Sample	Mean U/ml	CV %
1	7.0	5.0
2	33.8	4.9
3	78.3	6.3

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, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Crescendic glomerulonephritis	55	53	96.4
Morbus Wegener (cANCA pos)	20	1	5.0
Non-ANCA kidney disease	10	1	10.0
Normal human sera	120	3	2.5

		Immunological Diagnosis		
		POS	NEG	
ORG 519	POS	54	5	
	NEG	1	145	
		55	150	205

Sensitivity: 98.2 %
Specificity: 96.7 %
Overall agreement: 97.1 %

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 establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. Jennette, J. C. and Falk, R.J . Antineutrophil Cytoplasmic Autoantibodies and Associated Diseases: a Review. Am. J. Kidney Dis. 1990, Vol. XV, No. 6: 517 - 529.
2. Gross, W. L. et al. Antineutrophil Cytoplasmic Autoantibody-Associated Diseases: A Rheumatologist's Perspective. Am. J. Kidney Dis. 1991, Vol. XVIII, No. 2: 175 - 179.
3. Wieslander, J. How are Antineutrophil Cytoplasmic Autoantibodies Detected ? Am. J. Kidney Dis. 1991, Vol. XVIII, No. 2: 154 - 158.
4. Lesavre, P. Antineutrophil cytoplasmic antibodies antigen specificity. Am. J. Kidney Dis. 1991,Vol. XVIII,No. 2: 159 - 163.
5. Hagen, E. C. et al. Antineutrophil cytoplasmic autoantibodies: a review of the antigens involved, the assays, and the clinical and possible pathogenic consequences. Blood 1993, Vol.81: 1996 - 2000.
6. Gross, W .L. et al. Immunodiagnostische und immunopathogenetische Bedeutung von Anti-Neutrophilen-Cytoplasma-Antikörpern. Deutsche Medizinische Wochenschrift 1993, Vol. 118: 191 - 199.

Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 519_IFU_EN_QM113148_2016-05-03_1.3* Reason for revision: *Introduction electronic IFU on homepage*

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**

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529_4

ORG 529 Anti-Phospholipid Screen IgG/IgM

INTENDED PURPOSE

Anti-Phospholipid Screen IgG/IgM is an ELISA test system to screen for the presence of IgG and IgM class autoantibodies against cardiolipin, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid and 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.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
529_4	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE G	Enzyme Conjugate
CONJUGATE M	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

A mixture of highly purified cardiolipin, phosphatidyl serine, phosphatidyl inositol, phosphatidic acid and human 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 stopps 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.

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 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.

CONTENTS OF THE KIT

ORG 529	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***PSC***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 GPL-U/ml / 0 MPL-U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 6.3 GPL-U/ml / 6.3 MPL-U/ml, containing phospholipid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 12.5 GPL-U/ml / 12.5 MPL-U/ml, containing phospholipid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 25 GPL-U/ml / 25 MPL-U/ml, containing phospholipid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 50 GPL-U/ml / 50 MPL-U/ml, containing phospholipid antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 100 GPL-U/ml / 100 MPL-U/ml, containing phospholipid antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing phospholipid 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.
CONTROL −	1x 1.5 ml	Control negative, containing phospholipid 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.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE G	15 ml	Enzyme Conjugate; containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
CONJUGATE M	15 ml	Enzyme Conjugate; containing anti-human IgM antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

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.

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.

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 dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. 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.

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.

PREPARATION OF REAGENTS

WASH

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.

DILUENT

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.

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.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   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

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.

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.

PERFORMANCE CHARACTERISTICS

Calibration

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 GPL-U/ml IgM: 0 - 100 MPL-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: 10 GPL-U/ml IgM: 10 MPL-U/ml

Interpretation of results

Negative:	IgG < 10 GPL-U/ml	IgM < 10 MPL-U/ml
Positive:	≥ 10 GPL-U/ml	≥ 10 MPL-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 GPL/MPL-U/ml	Expected GPL/MPL-U/ml	O/E [%]
IgG 1	1:100	98.0	98.4	100
	1:200	49.6	49.2	101
	1:400	24.3	24.6	99
	1:800	12.0	12.3	98
	1:1600	5.8	6.2	94
IgG 2	1:100	92.4	92.4	100
	1:200	45.9	46.2	99
	1:400	22.7	23.1	98
	1:800	11.4	11.6	99
	1:1600	5.4	5.8	94
IgM 1	1:100	92.7	92.7	100
	1:200	45.7	46.4	99
	1:400	22.8	23.2	98
	1:800	11.2	11.6	97
	1:1600	5.4	5.8	93
IgM 2	1:100	72.4	74.2	100
	1:200	36.5	37.1	98
	1:400	18.7	18.6	101
	1:800	8.9	9.3	96
	1:1600	4.4	4.6	95

Limit of detection

Functional sensitivity was determined to be: IgG: 0.5 GPL-U/ml IgM: 0.5 MPL-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 GPL-U/ml	CV %
1	10.4	5.1
2	18.7	3.4
3	59.9	5.2

Inter-Assay IgG		
Sample	Mean GPL-U/ml	CV %
1	10.0	3.6
2	17.7	5.4
3	57.9	4.9

Intra-Assay IgM		
Sample	Mean MPL-U/ml	CV %
1	12.8	4.1
2	30.8	3.5
3	63.8	3.7

Inter-Assay IgM		
Sample	Mean MPL-U/ml	CV %
1	12.6	5.3
2	31.9	4.1
3	62.1	4.2

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, Heparine). 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	7	87.5	6	75.0
Secondary APS	65	60	92.3	33	50.8
Normal human sera	150	4	2.7	5	3.3

		Clinical Diagnosis POS	NEG	
ORG 529	POS	67	4	
IgG	NEG	6	146	
		73	150	223
Sensitivity:	91.8 %			
Specificity:	97.3 %			
Overall agreement:	95.5 %			

		Clinical Diagnosis Pos	Neg	
ORG 529	Pos	39	5	
IgM	Neg	34	145	
		73	150	223
Sensitivity:	53.4 %			
Specificity:	96.7 %			
Overall agreement:	82.5 %			

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 establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

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.

Notice to the user (European Union):

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control

Former version: *ORG 529_IFU_EN_QM113163_2016-04-18_3* Reason for revision: *Introduction electronic IFU on homepage*

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**

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538_3

ORG 538 ANAscreen

INTENDED PURPOSE

ANAscreen is an ELISA-based test system for the qualitative measurement of IgG class autoantibodies against SS-A 60, SS-A 52, SS-B, RNP-70, Sm, RNP/Sm, Scl-70, centromere B, Jo-1 in human serum or plasma samples. This product is intended for professional in vitro diagnostic use only.

The test is used for screening of patients with suspected autoimmune connective tissue diseases, e.g. systemic lupus erythematosus, mixed connective tissue disease, Sjoegren's syndrome, scleroderma, and polymyositis/dermatomyositis. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
538_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR	Calibrator
CONTROL –	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

A mixture of purified antigens SS-A 60, SS-A 52, SS-B, RNP-70, Sm, RNP/Sm, Scl-70, Centromere B and Jo-1 is coated on 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 stopps 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.

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 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.

CONTENTS OF THE KIT

ORG 538	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***Asc***
CALIBRATOR	1x 1.5 ml	Calibrator, containing ANA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL −	1x 1.5 ml	Control negative, containing ANA antibodies serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

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.

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.

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 dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production.
  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.

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.

PREPARATION OF REAGENTS

WASH

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.

DILUENT

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.

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.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µl** of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µl** of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   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	CAL											
B	C-											
C	P1											
D	P2											
E	P3											
F												
G												
H												

P1, ... patient sample CAL calibrator C- Control negative

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.

CALCULATION OF RESULTS

First optical density (OD) of cut-off is calculated by multiplying optical density of the calibrator by the test specific factor 0.5:

OD cut-off = OD Calibrator * 0.5

Then the optical density of a sample is compared to the optical density of the cut-off:

Negative: OD sample < OD cut-off
Positive: OD sample ≥ OD cut-off

For detailed results the optical density of a sample is expressed as Index value:

Index = OD sample / OD cut-off

PERFORMANCE CHARACTERISTICS

Calibration

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA.

Measuring range

not applicable

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 Index 1.0

Interpretation of results

Negative:	Index < 1.0
Borderline:	Index 1.0 - 1.2
Positive:	Index > 1.2

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer. Activity for each dilution step was calculated as Index-Value.

Sample	Dilution	Observed Index	Expected Index	O/E [%]
1	1:100	5.8	5.8	100
.	1:200	2.7	2.9	93
.	1:400	1.6	1.5	110
.	1:800	0.8	0.7	110
.	1:1600	0.4	0.4	106
2	1:100	4.9	4.9	100
.	1:200	2.7	2.5	110
.	1:400	1.3	1.2	106
.	1:800	0.6	0.6	98
.	1:1600	0.3	0.3	90

Limit of detection

not applicable not applicable

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		
Sample	Mean Index	CV %
1	1.1	3.5
2	1.9	2.4
3	3.2	2.2

Inter-Assay		
Sample	Mean Index	CV %
1	1.2	6.5
2	1.9	4.0
3	3.3	3.8

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, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
SLE	63	60	95.2
Sjogren's syndrome	10	10	100.0
MCTD	10	10	100.0
Poly-/dermatomyositis	8	7	87.5
Scleroderma	10	10	100.0
CREST syndrome	9	9	100.0
Normal human sera	148	3	2.0

		Clinical Diagnosis		
		POS	NEG	
ORG 538	POS	106	3	
	NEG	4	145	
		110	148	258

Sensitivity:	96.4	%
Specificity:	98.0	%
Overall agreement:	97.3	%

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 establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. Alba P, Bento L, Cuadrado MJ, Karim Y, Tungekar MF, Abbs I et al. Anti-dsDNA, anti-Sm antibodies, and the lupus anticoagulant: significant factors associated with lupus nephritis. Ann Rheum Dis 2003; 62(6):556-560.
2. Antico A, Platzgummer S, Bassetti D, Bizzaro N, Tozzoli R, Villalta D. Diagnosing systemic lupus erythematosus: new-generation immunoassays for measurement of anti-dsDNA antibodies are an effective alternative to the Farr technique and the Crithidia luciliae immunofluorescence test. Lupus 2010; 19(8):906-912.
3. Brouwer R, Hengstman GJ, Vree EW, Ehrfeld H, Bozic B, Ghirardello A et al. Autoantibody profiles in the sera of European patients with myositis. Ann Rheum Dis 2001; 60(2):116-123.
4. Castro C, Gourley M. Diagnostic testing and interpretation of tests for autoimmunity. J Allergy Clin Immunol 2010; 125(2 Suppl 2):S238-S247.
5. Defendenti C, Atzeni F, Spina MF, Grosso S, Cereda A, Guercilena G et al. Clinical and laboratory aspects of Ro/SSA-52 autoantibodies. Autoimmun Rev 2011; 10(3):150-154.
6. Eriksson C, Kokkonen H, Johansson M, Hallmans G, Wadell G, Rantapaa-Dahlqvist S. Autoantibodies predate the onset of Systemic Lupus Erythematosus in northern Sweden. Arthritis Research & Therapy 2011; 13(1):R30.
7. Haugbro K, Nossent JC, Winkler T, Figenschau Y, Rekvig OP. Anti-dsDNA antibodies and disease classification in antinuclear antibody positive patients: the role of analytical diversity. Ann Rheum Dis JID - 0372355 2004; 63 (4):386-394.
8. Ippolito A, Wallace DJ, Gladman D, Fortin PR, Urowitz M, Werth V et al. Autoantibodies in systemic lupus erythematosus: comparison of historical and current assessment of seropositivity. Lupus 2011; 20(3):250-255.
9. Isenberg DA, Manson JJ, Ehrenstein MR, Rahman A. Fifty years of anti-ds DNA antibodies: are we approaching journey's end? Rheumatology (Oxford) 2007; 46(7):1052-1056.
10. Kattah NH, Kattah MG, Utz PJ. The U1-snRNP complex: structural properties relating to autoimmune pathogenesis in rheumatic diseases. Immunol Rev 2010; 233(1):126-145.
11. Kumar Y, Bhatia A, Minz RW. Antinuclear antibodies and their detection methods in diagnosis of connective tissue diseases: a journey revisited. Diagn Pathol 2009; 4:1.
12. Meroni PL, Schur PH. ANA screening: an old test with new recommendations. Ann Rheum Dis 2010; 69:1420 -1422.
13. Petri M, Magder L. Classification criteria for systemic lupus erythematosus: a review. Lupus 2004; 13(11):829 -837.
14. Poole BD, Schneider RI, Guthridge JM, Velte CA, Reichlin M, Harley JB et al. Early targets of nuclear RNP humoral autoimmunity in human systemic lupus erythematosus. Arthritis Rheum 2009; 60(3):848-859.
15. Putova I, Dostal C, Becvar R. Prevalence of antinucleosome antibodies by enzyme-linked immunosorbent assays in patients with systemic lupus erythematosus and other autoimmune systemic diseases. Ann N Y Acad Sci 2007; 1109:275-286.
16. Reveille JD. Predictive value of autoantibodies for activity of systemic lupus erythematosus. Lupus JID - 9204265 2004; 13(5):290-297.
17. Simon JA, Cabiedes J, Ortiz E, Alcocer-Varela J, Sanchez-Guerrero J. Anti-nucleosome antibodies in patients with systemic lupus erythematosus of recent onset. Potential utility as a diagnostic tool and disease activity marker. Rheumatology (Oxford) 2004; 43(2):220-224.
18. Sinclair D, Saas M, Williams D, Hart M, Goswami R. Can an ELISA replace immunofluorescence for the detection of anti-nuclear antibodies?--The routine use of anti-nuclear antibody screening ELISAs. Clin Lab 2007; 53(3-4):183-191.
19. Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. Am J Clin Pathol 2002; 117(2):316-324.
20. Maidhof W., Hilias O. Lupus: an pverview of the disease and management options. P T 2012; 37(4):240-9.
21. Hahn BH, McMahon MA, Wilkinson A, Wallace WD, Daikh DI, Fitzgerald JD et al. American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. Arthritis Care Res (Hoboken) 2012; 64(6):797-808.

Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 538_IFU_EN_QM113172_2013-12-16_1.2* Reason for revision: *Introduction electronic IFU on homepage*

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**

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548_3

ORG 548 Anti-MCV

INTENDED PURPOSE

Anti-MCV is an ELISA test system for the quantitative measurement of IgG class autoantibodies against mutated citrullinated vimentin (MCV) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Measurement of anti-MCV antibodies contributes to early diagnosis of rheumatoid arthritis (RA), where anti-MCV antibody levels represent one parameter of a multi-criterion diagnostic process, encompassing both clinical and laboratory-based assessments.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
548_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Mutated citrullinated vimentin (MCV) 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 stopps 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.

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 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.

CONTENTS OF THE KIT

ORG 548	Σ 96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***MCV***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 20 U/ml, containing MCV antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 40 U/ml, containing MCV antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 100 U/ml, containing MCV antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 300 U/ml, containing MCV antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 1000 U/ml, containing MCV antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing MCV 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.
CONTROL −	1x 1.5 ml	Control negative, containing MCV 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.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

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.

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.

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 dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. 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.

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.

PREPARATION OF REAGENTS

WASH

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.

DILUENT

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.

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.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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

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.

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.

PERFORMANCE CHARACTERISTICS

CALIBRATION

This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

Measuring range

The calculation range of this ELISA assay is 0 - 1000 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 20 U/ml

Interpretation of results

Negative: < 20 U/ml
Positive: ≥ 20 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 [%]
1	1:100	882.8	882.8	100
.	1:200	386.0	441.4	87
.	1:400	205.2	220.7	93
.	1:800	110.7	110.4	100
.	1:1600	52.2	55.2	95
.	1:3200	23.4	27.6	85
2	1:100	932.1	932.1	100
.	1:200	486.0	466.1	104
.	1:400	250.1	233.0	107
.	1:800	126.6	116.5	109
.	1:1600	61.7	58.3	106
.	1:3200	28.2	29.1	97
3	1:100	727.9	727.9	100
.	1:200	362.4	364.0	100
.	1:400	178.2	182.0	98
.	1:800	85.7	91.0	94
.	1:1600	47.1	45.5	104
.	1:3200	19.2	22.7	85

Limit of detection

Functional sensitivity was determined to be: 1 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		
Sample	Mean U/ml	CV %
1	22.7	6.2
2	118.8	6.4
3	548.1	4.6

Inter-Assay		
Sample	Mean U/ml	CV %
1	20.2	5.3
2	111.0	9.2
3	451.6	7.7

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, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Rheumatoid arthritis	490	398	81.2
Other diseases	522	14	2.7
Normal human sera	234	1	0.4

		Clinical Diagnosis		
		POS	NEG	
ORG 548	POS	398	15	
	NEG	92	741	
		490	756	1246

Sensitivity: 81.2 %
Specificity: 98.0 %
Overall agreement: 91.4 %

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 establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. F.Bobbio-Pallavicini, C.Alpini, R.Caporali, S.Avalle, S.Bugatti, C.Montecuccio. Autoantibody profile in rheumatoid arthritis during long-term infliximab treatment. Arthritis Res Ther 2004, 6:R264-R272 (DOI 10.11 86/ar1173)
2. E.R.Vossenaar, N.Deprés, E.Lapointe,A.van der Heijden, M.Lora,T.Senshu,W.J.van Venfooij, H.A.Ménard. Rheumatoid arthritis specific anti Sa antibodies target citrullinated vimentin. Arthritis Research & Therapie Vol.6 No.2
3. O.Vittecoq, S.Poplin, K.Krzanowska, F.Jouen-Beades, J.F.Ménard, A.Daragon, F.Tron, X.Loet. Rheumatoid Factor is the strongest predictor of radiological progression of rheumatoid arthritis in a three-year prospective study in community-recruited patients. Rheumatology 2003; 42:939-946
4. W.J. van Venrooij, J.M.Hazes, H.Visser. Anticitrullinated protein/peptide antibody and its role in the diagnosis and prognosis of early rheumatoid arthritis. The Netherland Journal of Medicine.
5. M.Escalon, F.J.Lópees-Longo, C.M. González, I.Monteagudo, M.Rodriguez-Mahou, R.Grau, L.Carreno. Anti-Sa Sera from patients with Rheumatoid Arthritis contain at least 2 different subpopulations of Anti-Sa antibodies. The Journal of Rheumatology 2002; 29:10 2053-60
6. Ch.Vincent, L.Nogueira, M.Sebba, S.Chapuy-Regaud, M.Arnaud, O.Letourneur, D.Rolland, B.Rounie, A. Cantagrel, M.Jolivet, G.Serre. Detecion of antibodies to dertermined recombinant tat filaggrin by Enzyme-Linked Immunosorbent Assay. Arthritis & Rheumatism Vo. 46, No.8, August 2002, pp. 2051 -58
7. G.Steiner, J.Smolen. Antibodies in rheumatoid arthritis and their clinical significance. Arthritis Res 2002,4 (suppl 2):S1-S5
8. R.Goldbach-Mansky, J.Lee, A.McCoy, J.Hoxworth, C.Yarboro, J.S.Smolen, G.Steiner, A.Rosen, C.Zhang, H.A. Ménard, Z.J.Zhou, T.Palosuo, W.J.Van Venrooij, R.L.Wilder, J.H.Klippel, H.R.Schumacher Jr., H.S.El-Gabalawy. Rheumatoid arthritis associated antibodies in pati-ents with synovitis of recent onset. Arthritis Res 2000,2:236–243
9. H.Ménard, E.Lapointe, M.D.Rochdi, Z.J.Zhou. Insights into rheumatoid arthritis derived from the Sa immune system. Arthritis Research 2000,2:429-432
10. G.Hayem, P.Chazerain, B.Combe, A.Elias, T.Haim, P.Nicaise, K.Benali, J-F Eliau, M-F Kahn, J.Sany, O.Meyer. Anti-Sa antibody is an accurate diagnostic and prognostic Marker in adult Rheumatoid Arthritis.The Journal of Rheumatology 1 999;26:7-1 3
11. N. Deprés, G.Boire, F.J. Lopez-Longo, H.A. Ménard. The Sa System : A novel antigen antibo-dy system specific for rheumatoid arthritis. The Journal of Rheumatology 1994; 21 -:1 027- 33
12. E.R.Vossenaar,T.R.D. Radstake,A. van der Heijden, M.A.M. van Mansum, C. Dieteren, D.-J. de Rooij, P. Barrera, A.J.W. Zendman,W.J. van Venrooij. Expression and activity of citrullinatin peptidylarginine deiminase enzymes in monocytes and macrophages. Ann Rheum Dis 2004; 63:373-381
13. H. Burkhardt, B. Sehnert, R. Bockermann, A. Engström, J.R. Kalden, R. Holmdahl. Humoral immune response to citrullinated collagen type II determinants in early rheumatoid arthritis. Eur. J. Immunol. 2005.35: 1643-1652
14. Ch.Vincent, L. Nogueira, C. Clavel, M. Sebbag, G. Serre. Autoantibodies to citrullinated pro-teins: ACPA. Autoimmunity, February 2005; 38 (1): 17-24
15. M. Sebbag, S. Chapuy-Regaud, I. Auger, E. Petit-Texeira, C. Clavel, L. Nogueira, Ch.Vincent, F. Cornélis,J. Roudier, G. Serre.Clinical and pathophysiological significance of the autoimmu¬ne response to citrullinated proteins in rheumatoid arthritis. Joint bone Spine 71 (2004) 493-502

References Concerning Classification of RA

16. Liao KP, Batra KL, Chibnik L, Schur PH, Costenbader KH. Anti-CCP revised criteria for the classification of rheumatoid arthritis. Ann Rheum Dis 2008.
17. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988; 31(3):315 -24.Further Reading on Diagnostic Performance of Anti-MCV vs. Predicate Assay:
18. Gyetvai A, Szekanecz Z, Soos L, Szabo Z, Fekete A, Kapitany A et al. New classification of the shared epitope in rheumatoid arthritis: impact on the production of various anti-citrullinated protein antibodies. Rheumatology (Oxford) 2010; 49(1):25-33.
19. Pruijn G, Wiik A, van Venrooij W. The use of citrullinated peptides and proteins for the diagnosis of rheumatoid arthritis. Arthritis Research & Therapy 2010; 12(1):203.
20. Aggarwal R, Liao K, Nair R, Ringold S, Costenbander KH. Anti-citrullinated peptide antibody assays and their role in the diagnosis of rheumatoid arthritis. Athritis Rheum 2009; 61(11):1472-83.
21. Feitsma AL, van der Voort EI, Franken KL, El BH, Elferink BG, Drijfhout JW et al. Identification of citrullinated vimentin peptides as T cell epitopes in HLA-DR4-positive patients with rheumatoid arthritis. Arthritis Rheum 2009; 62(1):117-25.
22. Klareskog L, Catrina AI, Paget S. Rheumatoid arthritis. Lancet 2009; 373(9664):659-72.
23. Liu X, Jia R, Zhao J, Li Z. The Role of Anti-Mutated Citrullinated Vimentin Antibodies in the Diagnosis of Early Rheumatoid Arthritis. J Rheumatol 2009.
24. Luime JJ, Colin EM, Hazes JM, Lubberts E. Does anti-MCV has additional value as serological marker in the diagnostic and prognostic work-up of patients with rheumatoid arthritis? A systematic review. Ann Rheum Dis 2009.
25. Mjaavatten MD, Uhlig T, Haugen AJ, Nygaard H, Sidenvall G, Helgetveit K et al. Positive anti-citrullinated protein antibody status and small joint arthritis are consistent predictors of chronic disease in patients with very early arthritis: results from the NOR-VEAC cohort. Arthritis Res Ther 2009; 11(5):R146.
26. Engelmann R, Brandt J, Eggert M, Karberg K, Krause A, Neeck G et al. The Anti-mutated Citrullinated Vimentin Response Classifies Patients with Rheumatoid Arthritis into Broad and Narrow Responders. J Rheumatol 2009.
27. Raza K, Mathsson L, Buckley CD, Filer A, Ronnelid J. Anti-modified citrullinated vimentin (MCV) antibodies in patients with very early synovitis. Ann Rheum Dis 2009.
28. Snir O, Widhe M, Hermansson M, von SC, Lindberg J, Hensen S et al. Antibodies to several citrullinated antigens are enriched in the joints of rheumatoid arthritis patients. Arthritis Rheum 2009; 62(1):44-52.
29. Syversen SW, Goll GL, van der Heijde D, Landewe R, Lie BA, Odegard S et al. Prediction of radiographic progression in rheumatoid arthritis and the role of antibodies against mutated citrullinated vimentin: results from a ten-year prospective study. Ann Rheum Dis 2009.
30. van der Linden MP, van der Woude D, Ioan-Facsinay A, Levarht EW, Stoeken-Rijsbergen G, Huizinga TW et al. Value of anti-modified citrullinated vimentin and third-generation anti-cyclic citrullinated peptide compared with second-generation anti-cyclic citrullinated peptide and rheumatoid factor in predicting disease outcome in undifferentiated arthritis and rheumatoid arthritis. Arthritis Rheum 2009; 60(8):2232-41.
31. Wagner E, Skoumal M, Bayer PM, Klaushofer K. Antibody against mutated citrullinated vimentin: a new sensitive marker in the diagnosis of rheumatoid arthritis. Rheumatol Int 2009.
32. Keskin G, Inal A, Keskin D, Pekel A, Baysal O, Dizer U et al. Diagnostic Utility of Anti-Cyclic Citrullinated Peptide and Anti-Modified Citrullinated Vimentin Antibodies in Rheumatoid Arthritis. Protein Pept Lett 2008; 15 (3):314-7.
33. Szekanecz Z, Lakos G. Rheumatoid arthritis diagnosis with antimutated citrullinated vimentin ELISA by Orgentec Diagnostika. Exp Opin Med Diagnostics 2008; 2(9):1083-90.
34. Szekanecz Z, Soos L, Szabo Z, Fekete A, Kapitany A, Vegvari A et al. Anti-Citrullinated Protein Antibodies in Rheumatoid Arthritis: As Good as it Gets? Clin Rev Allergy Immunol 2008; 34(1):26-31.

Notice to the user (European Union):
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Change Control
Former version: *ORG 548_IFU_EN_QM113183_2013-12-16_1.2* Reason for revision: *Introduction electronic IFU on homepage*

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**

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601_3

ORG 601 Anti-CCP hs® (high sensitive)

INTENDED PURPOSE

Anti-CCP hs® (high sensitive) is an ELISA test system for the quantitative measurement of IgG class autoantibodies against cyclic citrullinated peptides (CCP) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

Measurement of anti-CCP antibodies may aid in the diagnosis of rheumatoid arthritis (RA), where anti-CCP antibody levels represent one parameter of a multi-criterion diagnostic process, encompassing both clinical and laboratory-based assessments.

SYMBOLS USED ON LABELS

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
96	Sufficient for 96 determinations
LOT	Batch code
	Use by
2°C 8°C	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult instructions for use
601_3	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	Calibrator
CALIBRATOR F	Calibrator
CONTROL +	Control positive
CONTROL −	Control negative
DILUENT	Sample Buffer P
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use

PRINCIPLE OF THE TEST

Highly purified cyclic citrullinated vimentin peptides (CCP) 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 stopps 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.

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 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.

CONTENTS OF THE KIT

ORG 601	96	Sufficient for 96 determinations
MICROPLATE	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Product code on module: ***CCP***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 U/ml, containing serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 20 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 40 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 100 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR E	1x 1.5 ml	Calibrator E 300 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 1000 U/ml, containing CCP antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing CCP 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.
CONTROL −	1x 1.5 ml	Control negative, containing CCP 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.
DILUENT	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
CONJUGATE	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
TMB	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
STOP	15 ml	Stop solution; contains acid. Ready to use.
WASH	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

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.

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.

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 dessicated in the clip bag provided.
- Shelf life of the unopended test kit is 18 months from day of production. 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.

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.

PREPARATION OF REAGENTS

WASH

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.

DILUENT

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.

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.
   Incubate for **30 minutes** at room temperature (20-28 °C).
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
2. Dispense **100 µ**l of enzyme conjugate into each well.
   Incubate for **15 minutes** at room temperature.
   Discard the contents of the microwells and **wash 3 times** with **300 µl** of wash solution.
3. Dispense **100 µ**l of TMB substrate solution into each well.
   Incubate for **15 minutes** at room temperature
4. **Add 100 µl** of stop solution to each well of the modules
   Incubate for **5 minutes** at room temperature.
   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										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

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

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.

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.

PERFORMANCE CHARACTERISTICS

Calibration

This assay system is calibrated in relative arbitrary units. It is calibrated against an external anti-CCP Assay, since no international reference sera for RA diagnostic are available so far.

Measuring range

The calculation range of this ELISA assay is 0 - 1000 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 20 U/ml

Interpretation of results

Negative: < 20 U/ml
Positive: ≥ 20 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 [%]
1	1:100	950.2	950.2	100
	1:200	467.3	475.1	98
	1:400	245.4	237.6	103
	1:800	115.6	118.8	97
2	1:100	120.0	120.0	100
	1:200	60.5	60.0	101
	1:400	31.4	30.0	105
	1:800	14.2	15.0	95
	1:1600	7.3	7.5	97
3	1:100	321.3	321.3	100
	1:200	157.9	160.7	98
	1:400	96.4	80.3	120
	1:800	48.2	40.2	120

Limit of detection

Functional sensitivity was determined to be: 1 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		
Sample	Mean U/ml	CV %
1	13.0	7.8
2	144.5	9.9
3	250.6	13.6

Inter-Assay		
Sample	Mean U/ml	CV %
1	12.3	6.1
2	134.9	7.1
3	262.2	9.3

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, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Study results

Study population	n	n Pos	%
Rheumatoid arthritis	259	237	91.5
Other arthritis	22	6	27.3
Other rheumatic disease	37	1	2.7
Healthy controls	118	1	0.8

		Clinical Diagnosis		
		POS	NEG	
ORG 601	POS	237	8	
	NEG	22	169	
		259	177	436

Sensitivity: 91.5 %
Specificity: 95.5 %
Overall agreement: 93.1 %

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 establishe its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. Bang H, Egerer K, Gauliard A, et al. Mutation and citrullination modifies vimentin to a novel autoantigen for rheumatoid arthritis. Arthritis Rheum. 56(8):2503, 2007.
2. Aletaha D, et al. Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis & Rheumatism 62 (9):2569-2581, 2010.
3. Pruijn GJ, Wiik A, van Venrooij WJ. The use of citrullinated peptides and proteins for the diagnosis of rheumatoid arthritis. Arthritis Res Ther 12 (1):203, 2010.
4. Snir O, Widhe M, Hermansson M, von Spee C, Lindberg J, Hensen S, Lundberg K, Engstrom A, Venables PJ, Toes RE, Holmdahl R, Klareskog L, Malmstrom V. Antibodies to several citrullinated antigens are enriched in the joints of rheumatoid arthritis patients. Arthritis Rheum 62 (1):44-52, 2009.
5. Van Steendam K, Tilleman K, Deforce D. The relevance of citrullinated vimentin in the production of antibodies against citrullinated proteins and the pathogenesis of rheumatoid arthritis. Rheumatology (Oxford), 2011.
6. Van Steendam K, Tilleman K, De Ceuleneer M, De Keyser F, Elewaut D, Deforce D. Citrulli-nated vimentin as an important antigen in immune complexes from synovial fluid of rheumatoid arthritis patients with antibodies against citrullinated proteins. Arthritis Res Ther 12 (4):R132, 2010.
7. Syversen SW, Goll GL, van der Heijde D, Landewe R, Lie BA, Odegard S, Uhlig T, Gaarder PI, Kvien TK. Prediction of radiographic progression in rheumatoid arthritis and the role of antibodies against mutated citrullinated vimentin: results from a ten-year prospective study. Ann.Rheum.Dis. 69 (2):345-351, 2009.
8. Innala L, Kokkonen H,Eriksson C, Jidell E, Berglin E, Dahlqvst SR. Antibodies against mu-tated citrullinated vimentin are a better predictor of disease activity at 24 months in early rheumatoid arthritis than antibodies against cyclic citrullinated peptides. J Rheumatol. 35(6):1002, 2008.
9. Mathsson L, Mullazehi M, Wick MC, et al. Antibodies against citrullinated vimentin in rheumatoid arthritis: higher sensitivity and extended prognostic value concerning future radiographic progression as compared with antibodies against cyclic citrullinated peptides. Arthritis Rheum. 58(1):36, 2008.
10. Soós L, Szekanecz Z, Szabó Z, et al. Clinical evaluation of anti-mutated citrullinated vi-mentin by ELISA in rheumatoid arthritis. J Rheumatol. 34(8):1658, 2007.
11. Bizzaro N, Tonutti E, Tozzoli R, Villalta D. Analytical and diagnostic characteristics of 11 2nd- and 3rd-generation immunoenzymatic methods for the detection of antibodies to citrullinated proteins. Clin Chem. 53 (8):1527, 2007.
12. Coenen D, Verschueren P, Westhovens R, Bossuyt X. Technical and diagnostic perfor-mance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis of rheumatoid arthritis. Clin Chem. 53(3):498, 2007.
13. Dejaco C, Klotz W, Larcher H, Duftner C, Schirmer M, Herold M. Diagnostic value of antibodies against a modified citrullinated vimentin in rheumatoid arthritis. Arthritis Res Ther. 8(4):R119, 2006.
14. Keskin G, Inal A, Keskin D, et al. Diagnostic utility of anti-cyclic citrullinated peptide and anti-modified citrullinated vimentin antibodies in rheumatoid arthritis. Protein Pept Lett. 15(3):314 2008.
15. Poulsom H, Charles PJ. Antibodies to citrullinated vimentin are a specific and sensitive marker for the diagnosis of rheumatoid arthritis. Clin Rev Allergy Immunol. 34(1):4 2008.
16. Song JS, Park GB, Park AJ. Comparison of anti-mutated citrullinated vimentin with anti-cyclic citrullinated peptide and rheumatoid factors for the diagnostic value of rheuma-toid arthritis [in Korean]. J Korean Rheum Assoc. 14(3):235 2007.

17. Ursum J, Nielen MM, van Schaardenburg D, et al. Antibodies to mutated citrullinated vimentin and disease activity score in early arthritis: a cohort study. Arthritis Res Ther. 10(1):R12, 2008.
18. Besada E, Nikolaisen C, Nossent H. Diagnostic value of antibodies against mutated citrullinated vimentin for rheumatoid arthritis. Clin Exp Rheumatol. 29(1):85 2011.

Notice to the user (European Union):
Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established .

Change Control
Former version: *ORG 601_IFU_EN_QM113201_2016-04-18_2* Reason for revision: *Introduction electronic IFU on homepage*

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**