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538_4

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IVD

REF 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
Σ	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
538_4	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
50 x	50 x concentrate

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	▽E 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.

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

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.

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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_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und
   3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und
   3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

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604_5
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IVD

REF ORG 604 Anti-dsDNA

INTENDED PURPOSE

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

The test is used as an aid in the differential diagnosis of inflammatory autoimmune diseases, especially systemic lupus erythematosus (SLE). Autoantibodies to dsDNA are diagnostic markers for SLE and levels may be elevated during active disease. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

SYMBOLS USED

IVD	In vitro diagnostic medical device
	Manufacturer
REF	Catalogue number
	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
604_5	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
CONJUGATE	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use
50 x	50 x concentrate

PRINCIPLE OF THE TEST

Highly purified double-stranded DNA (dsDNA) 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 604	▽ 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: ***dsD***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 IU/ml, containing no serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR B	1x 1.5 ml	Calibrator B 12.5 IU/ml, containing dsDNA 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 IU/ml, containing dsDNAantibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR D	1x 1.5 ml	Calibrator D 50 IU/ml, containing dsDNA 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 IU/ml, containing dsDNA antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 IU/ml, containing dsDNA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing dsDNA 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 dsDNA 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 PD, 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.

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 PD 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 international reference preparation WHO Wo/80 for human anti-dsDNA IgG antibodies as 200 IU/ml.

Measuring range

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

Interpretation of results

Negative: < 20 IU/ml
Positive: ≥ 20 IU/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 IU/ml	Expected IU/ml	O/E [%]
1	1:100	104.2	104.2	100
.	1:200	50.6	52.1	97
.	1:400	24.9	26.1	95
.	1:800	11.2	13.0	86
2	1:100	135.3	135.3	100
.	1:200	68.9	67.7	102
.	1:400	35.2	33.8	104
.	1:800	18.2	16.9	108

Limit of detection

Functional sensitivity was determined to be: 1 IU/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 IU/ml	CV %
1	26.0	4.5
2	61.0	3.1
3	114.0	6.4

Inter-Assay		
Sample	Mean IU/ml	CV %
1	29.0	12.4
2	68.0	7.3
3	138.0	5.2

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	n pos	%
SLE	202	164	81.2
Other autoimmune diseases	33	1	3.0
Normal human sera	115	1	0.9

		Clinical Diagnosis POS	NEG	
ORG 604	POS	164	2	
	NEG	38	146	
		202	148	350

Sensitivity: 81.2 %
Specificity: 98.6 %
Overall agreement: 88.6 %

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.

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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 604_IFU_EN_QM113202_2021-02-16_4* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

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IVD

REF 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	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
518_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	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use
50 x	50 x concentrate

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	▽ 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.

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.

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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_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

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REF 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
	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
512_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	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use
50 x	50 x concentrate

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	▽ 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.

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.

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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_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und
   3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und
   3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

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REF ORG 509 Anti-SS-B

INTENDED PURPOSE

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

Antibodies against SS-B are used for the differential diagnosis of systemic inflammatory autoimmune diseases. Autoantibodies against the SS-B protein are usually found together with anti-SS-A in cases of Sjoegren's syndrome. 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
E	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
509_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	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use
50 x	50 x concentrate

PRINCIPLE OF THE TEST

Highly purified SS-B 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 509	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 SS-B 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 SS-B 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 SS-B 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 SS-B 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 SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing SS-B 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 SS-B 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.

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										
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	124.2	127.1	98
	1:200	62.4	34.8	98
	1:400	33.2	17.4	104
	1:800	16.1	8.7	101
2	1:100	104.4	104.4	100
	1:200	53.1	52.2	102
	1:400	27.6	26.1	106
	1:800	13.9	13.1	107

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	28.8	5.6
2	67.1	5.8
3	143.2	5.2

Inter-Assay		
Sample	Mean U/ml	CV %
1	24.5	11.0
2	70.5	6.9
3	157.6	4.1

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	%
Sjoegren´s syndrome	70	43	61.4
Rheumatoid arthritis	20	1	5.0
Normal human sera	100	4	4.0

		Clinical Diagnosis POS	NEG	
ORG 509	POS	43	5	
	NEG	27	115	
		70	120	190

Sensitivity: 61.4 %
Specificity: 95.8 %
Overall agreement: 83.2 %

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.

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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 509_IFU_EN_QM113136_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und
   3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und
   3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

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REF 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
	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
601_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	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use
50 x	50 x concentrate

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.

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										
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.

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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_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

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CE

IVD

REF ORG 511 Anti-RNP/Sm

INTENDED PURPOSE

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

Antibodies against the RNP/Sm complex are useful in the diagnosis of mixed connective tissue disorder (MCTD, Sharp syndrome) and related autoimmune diseases. Antibodies against the 70 kDa protein of this complex are a very specific marker for Sharp syndrome. The Sm proteins are recognised by antibodies that may occur in cases of mixed connective tissue disorder and systemic lupus erythematosus. 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	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
511_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	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use
50 x	50 x concentrate

PRINCIPLE OF THE TEST

Highly purified RNP/Sm 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 511	▽E 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 RNP/Sm 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 RNP/Sm 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 RNP/Sm 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 RNP/Sm 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 RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing RNP/Sm 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 RNP/Sm 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.

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	161.4	161.4	100
.	1:200	78.0	80.7	97
.	1:400	39.7	40.4	98
.	1:800	20.1	20.2	100
2	1:100	167.2	167.2	100
.	1:200	83.7	83.6	100
.	1:400	41.5	41.8	99
.	1:800	20.8	20.9	100

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	65.6	4.1
2	101.9	5.9
3	182.0	1.8

Inter-Assay		
Sample	Mean U/ml	CV %
1	33.3	4.2
2	109.0	3.1
3	176.8	2.9

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	%
SLE	70	37	52.9
MCTD	30	29	96.7
Rheumatoid arthritis	20	3	15.0
Normal human sera	100	2	2.0

		Clinical Diagnosis POS	NEG	
ORG 511	POS	66	5	
	NEG	34	115	
		100	120	220

Sensitivity: 66.0 %
Specificity: 95.8 %
Overall agreement: 82.3 %

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.

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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 511_IFU_EN_QM113138_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

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REF ORG 508 Anti-SS-A

INTENDED PURPOSE

Anti-SS-A is an ELISA test system for the quantitative measurement of IgG class autoantibodies against SS-A (52 and 60 kDa) in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

This test is useful for the differential diagnosis and monitoring of systemic rheumatic inflammatory autoimmune diseases. Autoantibodies against the two antigens SS-A 52 and SS-A 60 are predominantly found in cases of Sjoegren's syndrome. 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
	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
508_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	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use
50 x	50 x concentrate

PRINCIPLE OF THE TEST

Highly purified SS-A (52 and 60 kDa) 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 508	▽ 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 SS-A 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 SS-A 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 SS-A 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 SS-A 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 SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing SS-A 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 SS-A 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.

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	139.0	139.0	100
	1:200	67.9	69.5	98
	1:400	33.0	34.8	95
	1:800	17.2	17.4	99
2	1:100	161.6	161.6	100
	1:200	70.6	80.8	87
	1:400	39.2	40.4	97
	1:800	20.0	20.2	99

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	32.2	2.7
2	73.2	2.6
3	134.0	3.6

Inter-Assay		
Sample	Mean U/ml	CV %
1	33.8	6.4
2	71.3	6.2
3	133.1	1.1

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	%
Sjoegren´s syndrome	70	51	72.9
Normal human sera	100	7	7.0

		Clinical Diagnosis POS	NEG	
ORG 508	POS	51	7	
	NEG	19	93	
		70	100	170

Sensitivity:	72.9	%
Specificity:	93.0	%
Overall agreement:	84.7	%

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.

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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 508_IFU_EN_QM113135_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

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IVD

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REF ORG 633 Anti-Centromere B

INTENDED PURPOSE

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

The test is used as an aid in the differential diagnosis of inflammatory autoimmune diseases, e.g. CREST syndrome. 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
	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
633_4	Electronic Instruction For Use: version

MICROPLATE	Microplate
CALIBRATOR A	Calibrator
CALIBRATOR B	Calibrator
CALIBRATOR C	Calibrator
CALIBRATOR D	Calibrator
CALIBRATOR E	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
50 x	50 x concentrate

PRINCIPLE OF THE TEST

Recombinant centromere protein B 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 633	▽E 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: ***CEN***
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 10 U/ml, containing centromere B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR C	1x 1.5 ml	Calibrator C 30 U/ml, containing centromere B 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 centromere B 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 centromere B antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing centromere B 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 centromere B 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.

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	P2										
B	B	P3										
C	C											
D	D											
E	E											
F	C+											
G	C-											
H	P1											

P1, ... patient sample A-E 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 - 300 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 10 U/ml

Interpretation of results

Negative: < 10 U/ml
Positive: ≥ 10 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	136.8	136.8	100
.	1:200	67.1	68.4	98
.	1:400	35.2	34.2	103
.	1:800	16.9	17.1	99
2	1:100	285.0	285.0	100
.	1:200	139.2	142.5	98
.	1:400	73.5	71.3	103
.	1:800	37.0	35.6	104

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	15.2	5.4
2	122.0	4.4
3	220.0	4.7

Inter-Assay		
Sample	Mean U/ml	CV %
1	16.4	5.4
2	125.6	5.0
3	225.4	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	n Pos	%
CREST syndrome	32	31	96.9
Rheumatoid arthritis	20	1	5.0
Normal human sera	100	6	6.0

		Clinical Diagnosis POS	NEG	
ORG 633	POS	31	7	
	NEG	1	113	
		32	120	152

Sensitivity: 96.9 %
Specificity: 94.2 %
Overall agreement: 94.7 %

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.

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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 633_IFU_EN_QM113209_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

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516_5

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REF ORG 516 AMA-M2

INTENDED PURPOSE

AMA-M2 is an ELISA test system for the quantitative measurement of IgG class autoantibodies against mitochondrial M2 subtype antigen in human serum or plasma. This product is intended for professional in vitro diagnostic use only.

The test is used as an aid in the differential diagnosis of primary biliary cirrhosis (PBC). In patients with other autoimmune diseases occurrence of AMA antibodies may be related to the development or association of PBC. 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	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
516_5	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
50 x	50 x concentrate

PRINCIPLE OF THE TEST

Highly purified mitochondrial M2 subtype (PDC-E2, BCOADC-E2, OGDC-E2) antigen 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 516	∑ 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: ***AMA***
CALIBRATOR A	1x 1.5 ml	Calibrator A 0 IU/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 IU/ml, containing AMA-M2 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 IU/ml, containing AMA-M2 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 IU/ml, containing AMA-M2 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 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, NaN3 0.09%), yellow. Ready to use.
CALIBRATOR F	1x 1.5 ml	Calibrator F 200 IU/ml, containing AMA-M2 antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Ready to use.
CONTROL +	1x 1.5 ml	Control positive, containing AMA-M2 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 AMA-M2 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.

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 international reference preparation WHO 67/183 for AMA-M2 as 100 IU/ml.

Measuring range

The calculation range of this ELISA assay is 0 - 200 IU/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 10 IU/ml

Interpretation of results

Negative: < 10 IU/ml
Positive: ≥ 10 IU/ml

Linearity

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 IU/ml	Expected IU/ml	O/E [%]
WHO	1:100	108.5	100.0	109
.	1:200	51.2	50.0	102
.	1:400	25.2	25.0	101
.	1:800	12.8	12.5	102
.	1:1600	6.1	6.3	98
.	1:3200	3.1	3.1	99
1	1:100	49.5	49.5	100
.	1:200	25.0	24.8	101
.	1:400	12.2	12.4	99
.	1:800	5.9	6.2	95

Limit of detection

Functional sensitivity was determined to be: 1 IU/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 IU/ml	CV %
1	39.8	7.0
2	81.3	3.8
3	177.3	3.6

Inter-Assay		
Sample	Mean IU/ml	CV %
1	40.1	6.2
2	84.6	11.8
3	180.4	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	%
Primary biliary cirrhosis (PBC)	143	139	97.2
Rheumatoid Arthritis	60	1	1.7
Normal human sera	267	18	6.7

		Clinical Diagnosis POS	NEG	
ORG 516	POS	139	19	
	NEG	4	308	
		143	327	470

Sensitivity:	97.2	%
Specificity:	94.2	%
Overall agreement:	95.1	%

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.

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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 516_IFU_EN_QM113145_2018-01-02_4* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und 3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen

CE

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DCM144-2
Ed. 03/2022

Anti Cardiolipin Screen

per analisi di routine

Determinazione quantitativa degli autoanticorpi IgG o IgM contro la cardiolipina nel siero o plasma umano

IVD | **LOT** Vedere l'etichetta esterna | 2°C 8°C | Σ = 96 test | **REF DKO144**

1. SCOPO PREVISTO

Per uso diagnostico *in vitro*
Per uso professionale in laboratorio

Anti Cardiolipin Screen è un dispositivo diagnostico manuale *in vitro* destinato alla determinazione quantitativa di anticorpi di classe IgG e IgM diretti contro la cardiolipina nel siero o nel plasma umano. I risultati devono essere impiegati in associazione ad altri dati clinici e di laboratorio come ausilio nella diagnosi della sindrome da antifosfolipidi (APS).

2. RILEVANZA CLINICA

La cardiolipina è un fosfolipide caricato negativamente che si trova in genere nella membrana mitocondriale interna[1]. Gli autoanticorpi diretti contro la cardiolipina fanno parte di un gruppo noto come anticorpi antifosfolipidi che comprende gli autoanticorpi anti-β2 glicoproteina 1. La misurazione degli autoanticorpi anti-cardiolipina è considerata uno dei marcatori più importanti per sostenere la diagnosi della sindrome da antifosfolipidi (APS)[2,3].

L'APS è un disturbo autoimmune sistemico caratterizzato da una combinazione di trombosi arteriose e/o venose, complicanze della gravidanza, quali perdita fetale ricorrente, insieme a livelli elevati di anticorpi antifosfolipidi[4]. L'APS è stata descritta per la prima volta in pazienti con lupus eritematoso sistemico (LES), anche se successivamente è stato stabilito che il LES può essere indipendente da una malattia sottostante[5].

L'APS può presentarsi da sola (APS primaria o in associazione ad altre condizioni, come il LES) o come APS secondaria[6] (6). Tuttavia, è stato dimostrato che gli anticorpi anti-cardiolipina (aCL) possono essere rilevati in pazienti con LES che non sviluppano APS secondaria[7-9]. Inoltre, gli eventi tromboembolici sono la manifestazione clinica più comune dell'APS.

Gli anticorpi anti-cardiolipina possono riconoscere sia la cardiolipina sia porzioni del complesso proteina-fosfolipide β2 glicoproteina 1-cardiolipina[10,11].

Alcuni studi hanno indicato un'associazione di anticorpi IgG e IgM anti-cardiolipina[2,7,11-16], con eventi trombotici, mentre altri suggeriscono che questi siano correlati all'isotipo IgG, ma non all'IgM[6,10,17].

È stato dimostrato che gli anticorpi IgM aCL sono presenti in infezioni quali epatite C cronica, lebbra e sifilide, ma non sono direttamente coinvolti in eventi trombotici[17].

È probabile che la presenza di anticorpi antifosfolipidi, tra cui IgG e IgM aCL, costituisca il singolo fattore di rischio più riconoscibile nei casi di perdita della gravidanza ricorrente e complicanze ostetriche mediate dalla placenta tardive[4-6,11,14-15,18,19]. Laddove le pazienti possono presentare solo esiti avversi della gravidanza con eventi vascolari isolati o con manifestazioni sia ostetriche sia trombotiche[6]. È stato suggerito che gli anticorpi anti-β2-glicoproteina I – cardiolipina sono in grado di riconoscere l'antigene su tessuti placentari, inibendo la crescita e la differenziazione dei trofoblasti che possono infine causare una placentazione difettosa[20].

3. PRINCIPIO DEL METODO

Il test Anti Cardiolipin Screen permette di determinare gli autoanticorpi diretti contro il complesso cardiolipina-β2-glicoproteina attraverso due diverse curve di calibrazione e coniugati enzimatici (uno specifico per il test IgG, uno specifico per il test IgM) e una micropiastra. Il principio del metodo e la procedura di dosaggio sono gli stessi per entrambe le valutazioni. Utilizzare reagenti per IgG o reagenti per IgM a seconda dell'isotipo in esame.

Anti Cardiolipin Screen è un dosaggio immunometrico enzimatico (ELISA) a sandwich in due fasi in cui i campioni dei pazienti, i calibratori o i controlli sono incubati su piastre per microtitolazione rivestite con il complesso antigenico cardiolipina-β2 glicoproteina. Durante l'incubazione, gli anticorpi presenti nel campione di test si legano al complesso antigenico immobilizzato. Dopo l'incubazione, la separazione del legato dal libero viene eseguita con un semplice lavaggio della fase solida.

Una successiva incubazione si verifica con anti-IgM o anti-IgG umane coniugate con perossidasi di rafano (HRP), che si lega agli anticorpi immobilizzati. Viene eseguita un'ulteriore fase di lavaggio per rimuovere il coniugato in eccesso. Quindi, una soluzione di substrato cromogenico contenente TMB viene erogata nei pozzetti che reagisce con l'HRP coniugato e si sviluppa un colore blu che cambia in giallo quando viene aggiunta la soluzione di arresto (H_2SO_4). L'intensità del colore è direttamente proporzionale

alla concentrazione di IgM o IgG anti-cardiolipina (a seconda del coniugato utilizzato) nel campione.

La concentrazione di anticorpo anti-cardiolipina nel campione viene calcolata attraverso una curva di calibrazione.

4. REAGENTI, MATERIALI E STRUMENTAZIONE

4.1. Reagenti e materiali forniti nel kit

Per la determinazione di anticorpi di classe IgG

1. Calibratori di IgG anti-cardiolipina
(5 fiale, 1,2 mL ciascuno)
Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

CAL0	**REF DCE002/11306-0**
CAL1	**REF DCE002/11307-0**
CAL2	**REF DCE002/11308-0**
CAL3	**REF DCE002/11309-0**
CAL4	**REF DCE002/11310-0**

2. Controlli (2 fiale, 1,2 mL ciascuna, pronte all'uso)
Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

Controllo negativo	**REF DCE045/11301-0**
Controllo positivo	**REF DCE045/11302-0**

3. Coniugato IgG (1 fiala, 15 mL)
Coniugato anti h-IgG con perossidasi di rafano (HRP), BSA 0,1%, ProClin < 0,0015% **REF DCE002/11302-0**

Per la determinazione di anticorpi di classe IgM

1. Calibratori (5 fiale, 1,2 mL ciascuno)
Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

CAL0	**REF DCE002/11206-0**
CAL1	**REF DCE002/11207-0**
CAL2	**REF DCE002/11208-0**
CAL3	**REF DCE002/11209-0**
CAL4	**REF DCE002/11210-0**

2. Controlli (2 fiale, 1,2 mL ciascuna, pronte all'uso)
Tampone fosfato 0,1 M, NaN_3 < 0,1%, siero umano

Controllo negativo	**REF DCE045/11201-0**
Controllo positivo	**REF DCE045/11202-0**

3. Coniugato (1 fiala, 15 mL)
Coniugato anti h-IgM con perossidasi di rafano (HRP), BSA 0,1%, ProClin < 0,0015% **REF DCE002/11202-0**

Reagenti comuni

4. Diluente per campione (1 fiala, 100 mL)
Tampone fosfato 0,1 M, NaN_3 < 0,1%
REF DCE053-0

5. Micropiastra rivestita (1 micropiastra frangibile)
Micropiastra rivestita con complesso antigenico cardiolipina-β2-glicoproteina **REF DCE002/14403-0**

6. Substrato TMB (1 fiala, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(evitare qualsiasi contatto con la pelle)*
REF DCE004-0

7. Soluzione di arresto (1 fiala, 15 mL)
Acido solforico 0,15 M *(evitare qualsiasi contatto con la pelle)*
REF DCE005-0

8. Soluzione di lavaggio conc. 10X (1 fiala, 50 mL)
Tampone fosfato 0,2 M, pH 7,4 **REF DCE054-0**

4.2. Materiali richiesti ma non forniti

Acqua distillata

4.3. Materiali e strumentazione ausiliari

Erogatore automatico
Pipette di precisione
Lettore di micropiastre (450 nm, 620-630 nm)

5. AVVERTENZE

- Questo kit è destinato all'uso *in vitro* esclusivamente da parte di professionisti. Non per uso interno o esterno in esseri umani o animali.
- Utilizzare adeguati dispositivi di protezione individuale mentre si lavora con i reagenti forniti.
- Seguire le buone prassi di laboratorio (GLP, Good Laboratory Practice) per la manipolazione di emoderivati.
- Tutto il materiale di origine umana utilizzato nella preparazione dei reagenti è stato testato e risultato negativo per gli anticorpi dell'HIV 1 e 2, HbsAg e HCV. Nessun metodo di prova, tuttavia, può offrire la completa garanzia che HIV, HBV, HCV o altri agenti infettivi siano assenti. Pertanto, i calibratori e i controlli devono essere manipolati allo stesso modo del materiale potenzialmente infettivo.
- Il materiale di origine animale utilizzato nella preparazione del kit è stato ottenuto da animali certificati come sani e la proteina bovina è stata ottenuta da Paesi non infettati dalla BSE, ma tali materiali devono essere trattati come potenzialmente infettivi.
- Alcuni reagenti contengono piccole quantità di azoturo di sodio (NaN_3) o ProClin™ 300 come conservante. Evitare il contatto con pelle o mucose.
- L'azoturo di sodio può essere tossico se ingerito o assorbito attraverso la pelle o gli occhi; inoltre, può reagire con le tubature di piombo o rame per formare azoturi metallici potenzialmente esplosivi. Se si utilizza un lavandino per rimuovere i reagenti, lavare con abbondante acqua per evitare l'accumulo di azoturi.
- Il substrato TMB contiene un irritante, che può essere dannoso se inalato, ingerito o assorbito per via cutanea. Per prevenire lesioni, evitare l'inalazione, l'ingestione o il contatto con pelle e occhi.
- La soluzione di arresto consiste in una soluzione diluita di acido solforico. L'acido solforico è velenoso e corrosivo e può essere tossico se ingerito. Per prevenire ustioni chimiche, evitare il contatto con pelle e occhi.
- Evitare l'esposizione del reagente TMB/H_2O_2 a luce solare diretta, metalli o ossidanti. Non congelare la soluzione.

6. PRECAUZIONI

- Attenersi rigorosamente alla sequenza dei passaggi di pipettaggio forniti in questo protocollo. I dati sulle prestazioni qui rappresentati sono stati ottenuti utilizzando i reagenti specifici elencati in queste istruzioni per l'uso.
- Tutti i reagenti devono essere conservati refrigerati a 2-8 °C nel contenitore originale. Tutte le eccezioni sono chiaramente indicate.
- Lasciare che tutti i componenti del kit e i campioni raggiungano la temperatura ambiente (22-28 °C) e mescolare bene prima dell'uso.
- Non scambiare i componenti di kit di lotti diversi. La data di scadenza stampata sulle etichette della confezione e delle fiale deve essere rispettata. Non utilizzare alcun componente del kit dopo la data di scadenza.

- **AVVERTENZA: il reagente coniugato è progettato per garantire la massima sensibilità per la dose e può essere contaminato da agenti esterni se non utilizzato correttamente;** pertanto, si raccomanda di utilizzare materiali di consumo monouso (puntali, flaconi, vassoi, ecc.). Per le dosi divise, prelevare l'esatta quantità di coniugato necessaria e non reintrodurre alcun prodotto di scarto nel flacone originale. Inoltre, **per le dosi erogate con l'ausilio di dispositivi automatici e semiautomatici,** prima di utilizzare il coniugato, è consigliabile pulire il sistema per la gestione dei fluidi, assicurandosi che le procedure di lavaggio, deproteinizzazione e decontaminazione siano efficaci per evitare la contaminazione del coniugato; questa procedura è altamente raccomandata quando il kit viene elaborato con analizzatori non dotati di puntali monouso.
  A tale scopo, DiaMetra fornisce un reagente di decontaminazione separato per la pulizia degli aghi.
- Se si utilizzano apparecchiature automatizzate, l'utente ha la responsabilità di assicurarsi che il kit sia stato adeguatamente testato.
- La rimozione incompleta o imprecisa del liquido dai pozzetti potrebbe influenzare la precisione del dosaggio e/o aumentare il background. Per migliorare le prestazioni del kit sui sistemi automatici, si raccomanda di aumentare il numero di lavaggi.
- È importante che il tempo di reazione in ogni pozzetto sia mantenuto costante per ottenere risultati riproducibili. Il pipettaggio dei campioni non deve andare oltre i dieci minuti per evitare deviazioni del dosaggio. Se sono necessari più di 10 minuti, seguire lo stesso ordine di erogazione. Se si utilizza più di una piastra, si raccomanda di ripetere la curva dose-risposta in ogni piastra.
- L'aggiunta della soluzione di substrato TMB avvia una reazione cinetica, che viene terminata dall'aggiunta della soluzione di arresto. Pertanto, il substrato TMB e la soluzione di arresto devono essere aggiunti nella stessa sequenza per eliminare qualsiasi deviazione temporale durante la reazione.
- Osservare le linee guida per l'esecuzione del controllo di qualità nei laboratori medici analizzando i controlli e/o i sieri in pool.
- La massima precisione è richiesta per la ricostituzione e l'erogazione dei reagenti.
- I campioni microbiologicamente contaminati, altamente lipemici o emolizzati non devono essere utilizzati nel dosaggio.
- I lettori di piastre misurano verticalmente. Non toccare il fondo dei pozzetti.

7. CONSERVAZIONE E STABILITÀ DEI REAGENTI

Conservare il kit a 2-8 °C, al buio.

- Il kit è stabile a 2-8 °C fino alla data di scadenza indicata sull'etichetta esterna del kit.
- Una volta aperto, il kit è stabile a 2-8 °C per 6 mesi.
- La soluzione di lavaggio diluita è stabile per 30 giorni a 2-8 °C.

Nota importante: aprire il sacchetto contenente la micropiastra rivestita solo quando è a temperatura ambiente e chiuderlo immediatamente dopo l'uso.

8. RACCOLTA E CONSERVAZIONE DEI CAMPIONI

Il dosaggio deve essere effettuato su campioni di siero (provette di campionamento standard o provette contenenti gel per la separazione del siero) o plasma (litio eparina, sodio eparina o EDTA di potassio).

Conservazione dei campioni	Durata
2-8 °C	96 ore
Cicli di congelamento/scongelamento	3 cicli

9. PROCEDURA

9.1. Preparazione di calibratori e controlli

I calibratori e i controlli sono pronti per l'uso.
I calibratori presentano all'incirca le seguenti concentrazioni:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

9.2. Preparazione della soluzione di lavaggio

Diluire il contenuto della fiala "Soluzione di lavaggio conc. 10X" con acqua distillata fino a un volume finale di 500 mL prima dell'uso. Per i volumi più piccoli, rispettare il rapporto di diluizione 1:10.

È possibile osservare la presenza di cristalli all'interno della soluzione di lavaggio concentrata; in tal caso, mescolare a temperatura ambiente fino alla completa dissoluzione dei cristalli. Per una maggiore precisione, diluire l'intero flacone di soluzione di lavaggio concentrata a 500 mL, avendo cura anche di trasferire completamente i cristalli sciacquando il flacone, quindi mescolare fino a quando i cristalli non si dissolvono completamente.

9.3. Preparazione dei campioni

Tutti i campioni di siero e plasma devono essere diluiti 1:100 con diluente per campione.
ad es. 10 µL di campione devono essere diluiti con 990 µL di diluente per campione.

9.4. Procedura

- **Lasciare che tutti i reagenti raggiungano la temperatura ambiente (22-28 °C) per almeno 30 minuti.** Alla fine del dosaggio, conservare immediatamente i reagenti a 2-8 °C: evitare una lunga esposizione a temperatura ambiente.
- Le strisce di micropozzetti rivestiti non utilizzate devono essere rilasciate in modo sicuro nella busta di alluminio contenente l'essiccante e conservate a 2-8 °C.
- Per evitare potenziali contaminazioni microbiche e/o chimiche, i reagenti inutilizzati non devono mai essere trasferiti nelle fiale originali.
- Poiché è necessario eseguire la determinazione in duplicato per migliorare la precisione dei risultati del test, preparare due pozzetti per ogni punto della curva di calibrazione (C_0-C_4), due per ogni controllo, due per ogni campione, uno per il bianco.

La seguente procedura è la stessa per entrambi i test degli anticorpi di classe IgG e IgM.

Reagenti	Calibratore	Campione/ Controlli	Bianco
Utilizzare reagenti per IgG o reagenti per IgM a seconda dell'isotipo in esame			
Calibratore C_0-C_4 (IgG o IgM)	**100 µL**		
Controlli (IgG o IgM)		**100 µL**	
Campione diluito		**100 µL**	
Incubare per 60 minuti a temperatura ambiente (22-28 °C). Rimuovere il contenuto da ogni pozzetto; lavare i pozzetti 3 volte con 300 µL di soluzione di lavaggio diluita. **Nota importante**: durante ogni fase di lavaggio, agitare delicatamente la piastra per 5 secondi e rimuovere la soluzione in eccesso picchiettando la piastra capovolta su un tovagliolo di carta assorbente. **Lavatore automatico**: se si utilizzano apparecchiature automatiche, lavare i pozzetti almeno 5 volte.			
Coniugato (IgG o IgM)	**100 µL**	**100 µL**	
Incubare per 60 minuti a temperatura ambiente (22-28 °C). Rimuovere il contenuto da ogni pozzetto; lavare i pozzetti 3 volte con 300 µL di soluzione di lavaggio diluita. **Lavaggio**: seguire le stesse indicazioni del punto precedente.			
Substrato TMB	**100 µL**	**100 µL**	**100 µL**
Incubare per 15 minuti, al buio, a temperatura ambiente (22-28 °C).			
Soluzione di arresto	**100 µL**	**100 µL**	**100 µL**
Agitare delicatamente la micropiastra. Leggere l'assorbanza (E) a 450 nm contro una lunghezza d'onda di riferimento di 620-630 nm o contro il bianco entro 5 minuti.			

10. CONTROLLO QUALITÀ

Le buone prassi di laboratorio (GLP) richiedono l'inclusione di campioni per il controllo della qualità in ogni serie di dosaggi al fine di verificare le prestazioni del dosaggio. I controlli devono essere trattati come campioni sconosciuti e i risultati devono essere analizzati con metodi statistici appropriati.
I controlli forniti nel kit devono essere testati come se fossero sconosciuti e hanno lo scopo di agevolare la valutazione della validità dei risultati ottenuti in ogni piastra di dosaggio.

La concentrazione media di ciascun livello di controllo è documentata nel rapporto del controllo di qualità incluso in ciascun kit. Tali livelli di concentrazione media sono determinati in base a diversi dosaggi eseguiti in duplicato in più posizioni su ciascuna piastra. Questo test è valido solo se la densità ottica a 450 nm per i controlli e per i calibratori (C_0-C_4) rientra nel rispettivo intervallo indicato sul Certificato di controllo qualità allegato a ciascun kit del test.

DiaMetra raccomanda agli utenti di conservare le annotazioni grafiche dei valori di controllo generati con ciascun dosaggio, tra cui medie mobili, DS e CV%. Queste informazioni faciliteranno l'analisi delle tendenze dei controlli per quanto riguarda le prestazioni dei lotti di controllo attuali e pregressi rispetto ai dati forniti nel controllo di qualità. Le tendenze aiuteranno a identificare i dosaggi che generano valori di controllo significativamente diversi dal rispettivo intervallo medio.

Quando si interpretano i dati dei controlli, occorre tenere conto del fatto che il prodotto è stato progettato e sviluppato come prodotto per l'utilizzo manuale. L'intervallo riportato sul certificato del controllo di qualità deve essere appropriato per i dosaggi eseguiti manualmente e rispettando rigorosamente la procedura di dosaggio descritta sopra. Gli esperti del controllo di qualità riconoscono che, a causa delle differenze di condizioni e di prassi, si avrà sempre una variabilità nei valori medi e nella precisione delle misurazioni dei controlli eseguite da laboratori diversi[21].

11. CALCOLO DEI RISULTATI

Sono disponibili vari pacchetti software di elaborazione dei dati, che possono essere utilizzati per generare la curva di calibrazione media e per calcolare le concentrazioni medie di campioni e controlli sconosciuti. È necessario un adattamento della curva logistica a 4 parametri (4PL) con coordinate log-lineari **che includa il calibratore 0.** È possibile utilizzare un adattamento uniforme della curva spline che includa il calibratore 0. Gli altri algoritmi di adattamento della curva non sono raccomandati.

In alternativa, è possibile preparare una curva di calibrazione su carta millimetrata semilogaritmica tracciando un grafico con l'assorbanza media sull'asse delle ordinate e la concentrazione dell'analita sull'asse delle ascisse. Nella curva di calibrazione deve essere incluso il calibratore 0. Leggere il valore medio dell'assorbanza di ciascun campione sconosciuto dalla curva.

12. INTERVALLO DI MISURAZIONE

12.1. Per la valutazione di anticorpi di classe IgG

L'intervallo di misurazione del test (AMR) è 2-80 AU/mL. Qualsiasi valore inferiore a 2 AU/mL deve essere indicato come "< 2 AU/mL". Qualsiasi valore superiore a 80 AU/mL deve essere indicato come "> 80 AU/mL".

12.2. Per la valutazione di anticorpi di classe IgM

L'intervallo di misurazione del test (AMR) è 2,08-80 AU/mL. Qualsiasi valore inferiore a 2,09 AU/mL deve essere indicato come "< 2,08 AU/mL". Qualsiasi valore superiore a 80 AU/mL deve essere indicato come "> 80 AU/mL".

13. METROLOGIA E TRACCIABILITÀ

13.1. Per la valutazione di anticorpi di classe IgG

I calibratori di questo kit sono tracciabili al Centres for Disease Control (CDC) Human IgG Anti-Cardiolipin Monoclonal Antibody HCAL – Catalogue [IS2717].

13.2. Per la valutazione di anticorpi di classe IgM

I calibratori di questo kit sono tracciabili al Centres for Disease Control (CDC) Human IgM Anti-Cardiolipin Monoclonal Antibody EY2C9 [IS2718].

14. INTERPRETAZIONE DEI RISULTATI

Concentrazione	Interpretazione
< 8 AU/mL	Il campione deve essere considerato negativo
8-10 AU/mL	Il campione deve essere classificato equivoco e la ripetizione dei test/campionamenti deve essere eseguita secondo le pratiche interne
> 10 AU/mL	Il campione deve essere considerato positivo

La determinazione di un intervallo di valori attesi per una popolazione "normale" di un determinato metodo dipende da diversi fattori, come la specificità e la sensibilità del metodo utilizzato e il tipo di popolazione in esame. Pertanto, ogni laboratorio deve considerare l'intervallo fornito dal produttore come un'indicazione generale e produrre il proprio intervallo di valori attesi sulla base della popolazione autoctona.

I risultati positivi devono essere verificati in relazione all'intero stato clinico del paziente e la decisione per la terapia deve essere presa in base alle condizioni di ciascun paziente. È consigliabile che ogni laboratorio stabilisca i propri intervalli normali e patologici dei valori dell'anticorpo anti-cardiolipina.

15. CARATTERISTICHE DI AZIONE

Sono mostrati i dati più rappresentativi delle prestazioni. I risultati ottenuti nei singoli laboratori possono variare.

15.1. Per la valutazione di anticorpi di classe IgG

15.1.1. Capacità di rilevamento

Il limite del bianco (LoB), il limite di rilevamento (LoD) e il limite della determinazione quantitativa (LoQ) sono stati definiti basandosi sulla procedura CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" utilizzando 6 bianchi e 6 campioni a basso livello.

Sensibilità	Concentrazione
Limite del bianco (LoB)	0,59 AU/mL
Limite di rilevamento (LoD)	1,25 AU/mL
Limite della determinazione quantitativa (LoQ)	2,00 AU/mL

15.1.2. Esattezza

L'esattezza del test Anti Cardiolipin Screen per la valutazione di anticorpi di classe IgG è stata dimostrata attraverso l'esecuzione di un test di recupero utilizzando il CDC Human IgG Anti-Cardiolipin Monoclonal Antibody HCAL – Catalogue [IS2717].

15.1.3. Sensibilità e specificità diagnostica

La sensibilità e la specificità sono state determinate con CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" utilizzando 50 campioni negativi e 51 positivi eseguiti su due lotti di reagenti.

		DKO144 - IgG		Totale
		Positivo	Negativo	
Stato reale	Positivo	47	4	51
	Negativo	0	57	57
Totale		47	61	108

Sensibilità diagnostica: 92%

Specificità diagnostica: 100%

15.1.4. Precisione

La precisione del test Anti Cardiolipin Screen per la determinazione degli anticorpi di classe IgG è stata determinata eseguendo un complesso studio di precisione.

Ripetibilità: un totale di 6 campioni di siero è stato analizzato in 5 repliche, una volta al giorno per 5 giorni da 3 operatori.
I dati di un lotto rappresentativo sono mostrati di seguito:

Campione	n	Conc. media (AU/mL)	Intra-test (ripetibilità)	
			DS	CV
1	75	6,95	0,38	5,5%
2	75	11,03	0,51	4,6%
3	75	20,12	0,94	4,7%
4	75	30,26	1,86	6,1%
5	75	50,11	2,58	5,1%
6	75	71,71	2,53	3,5%

Riproducibilità: un totale di 6 campioni di siero è stato analizzato in 5 repliche, una volta al giorno per 5 giorni da 3 operatori.
I risultati per i dati combinati di due lotti sono mostrati di seguito:

Campione	n	Conc. media (AU/mL)	All'interno del laboratorio (riproducibilità)	
			DS	% CV
1	150	6,98	0,49	7,0%
2	150	11,17	0,84	7,5%
3	150	20,16	1,87	9,3%
4	150	30,38	3,06	10,1%
5	150	50,76	5,02	9,9%
6	150	72,30	3,93	5,4%

15.1.5. Linearità

La linearità è stata valutata secondo le linee guida basate su CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". Per la concentrazione di IgG anti-cardiolipina mediante il test Anti Cardiolipin Screen, la procedura di misurazione mostra linearità per l'intervallo

compreso tra 0,84 e 83,68 ng/mL entro la deviazione ammissibile di linearità (ADL) di ± 15%.

15.2. Specificità analitica

Le seguenti sostanze non interferiscono con un bias > ± 15% nel test Anti Cardiolipin Screen quando si valutano gli anticorpi di classe IgG se le concentrazioni sono inferiori alla soglia dichiarata presentata nella tabella seguente.

Reagente potenzialmente interferente	Concentrazione di soglia
Bilirubina, coniugata	15 mg/dL
Bilirubina, non coniugata	15 mg/dL
Emoglobina	200 mg/dL
Proteine totali	10 g/dL
Trigliceridi	500 mg/dL

15.2.1. Studio su siero-plasma

È stato condotto uno studio di confronto tra matrici del test Anti Cardiolipin Screen per la valutazione di anticorpi di classe IgG per valutare la differenza tra i tipi di provette (provette per la separazione del siero (SST), per plasma in litio eparina, per plasma in sodio eparina e plasma in K2 EDTA) rispetto ai campioni di controllo (siero tappo rosso, senza additivo) secondo le linee guida CLSI EP9-A3. È stato valutato un totale di 22 campioni (18 nativi, 4 additivati) per coprire l'intervallo. L'analisi di regressione lineare è stata effettuata su dati comparativi:

Tipo di campione	Pendenza [IC 95%]	Intercetta (ng/mL) [IC 95%]	Coefficiente di correlazione (r)
SST	0,96 [0,92-0,98]	0,64 [da -0,38 a 1,66]	1,00
Litio eparina	0,92 [da 0,88 a 0,96]	0,87 [da -0,24 a 1,99]	1,00
Sodio eparina	0,94 [da 0,89 a 0,98]	0,66 [da -0,75 a 2,06]	0,99
EDTA	0,95 [da 0,92 a 0,99]	0,54 [da -0,69 a 1,77]	1,00

15.3. Per la valutazione di anticorpi di classe IgM

15.3.1. Capacità di rilevamento

Il limite del bianco (LoB), il limite di rilevamento (LoD) e il limite della determinazione quantitativa (LoQ) sono stati definiti basandosi sulla procedura CLSI EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation" utilizzando 6 bianchi e 6 campioni a basso livello.

Sensibilità	Concentrazione
Limite del bianco (LoB)	0,76 AU/mL
Limite di rilevamento (LoD)	1,45 AU/mL
Limite della determinazione quantitativa (LoQ)	2,08 AU/mL

15.3.2. Esattezza

L'esattezza del test Anti Cardiolipin Screen per la valutazione di anticorpi di classe IgM è stata dimostrata attraverso l'esecuzione di un test di recupero utilizzando il CDC Human IgM Anti-Cardiolipin Monoclonal Antibody EY2C9 [IS2718].

15.3.3. Sensibilità e specificità diagnostica

La sensibilità e la specificità sono state determinate con CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" utilizzando 73 campioni negativi e 62 positivi eseguiti su due lotti di reagenti.

		DKO144 IgM		Totale
		Positivo	Negativo	
Stato reale	**Positivo**	50	12	**62**
	Negativo	0	73	**73**
Totale		**50**	**85**	**135**

Sensibilità diagnostica: 80%

Specificità diagnostica: 100%

15.3.4. Precisione

La precisione del test Anti Cardiolipin Screen per la determinazione degli anticorpi di classe IgM è stata determinata eseguendo un complesso studio di precisione.

Ripetibilità: un totale di 6 campioni di siero è stato analizzato in 5 repliche, una volta al giorno per 5 giorni da 3 operatori.
I dati di un lotto rappresentativo sono mostrati di seguito:

Campione	n	Conc. media (AU/mL)	Intra-test (ripetibilità)	
			DS	CV
1	75	7,74	0,40	5,2%
2	75	12,46	0,51	4,1%
3	75	21,06	0,99	4,7%
4	75	32,07	1,46	4,6%
5	75	55,15	1,19	2,2%
6	75	75,45	2,33	3,1%

Riproducibilità: un totale di 6 campioni di siero è stato analizzato in 5 repliche, una volta al giorno per 5 giorni da 3 operatori.
I risultati per i dati combinati di due lotti sono mostrati di seguito:

Campione	n	Conc. media (AU/mL)	All'interno del laboratorio (riproducibilità)	
			DS	% CV
1	150	7,65	0,46	6,0%
2	150	12,33	0,74	6,0%
3	150	21,03	1,60	7,6%
4	150	31,89	1,98	6,2%
5	150	54,28	2,70	5,0%
6	150	75,46	2,63	3,5%

15.3.5. Linearità
La linearità è stata valutata secondo le linee guida basate su CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". Per la concentrazione di IgM anti-cardiolipina mediante il test Anti Cardiolipin Screen, la procedura di misurazione mostra linearità per l'intervallo compreso tra 0,82 e 86,88 AU/mL entro la deviazione ammissibile di linearità (ADL) di ± 15%.

15.3.6. Specificità analitica
Le seguenti sostanze non interferiscono con un bias > ± 15% nel test Anti Cardiolipin Screen quando si valutano gli anticorpi di classe IgM se le concentrazioni sono inferiori alla soglia dichiarata presentata nella tabella seguente.

Reagente potenzialmente interferente	Concentrazione di soglia
Bilirubina, coniugata	15 mg/dL
Bilirubina, non coniugata	15 mg/dL
Emoglobina	200 mg/dL
Proteine totali	10 g/dL
Trigliceridi	500 mg/dL

15.3.7. Studio su siero-plasma
È stato condotto uno studio di confronto tra matrici del test Anti Cardiolipin Screen per la valutazione di anticorpi di classe IgM per valutare la differenza tra i tipi di provette (provette per la separazione del siero (SST), per plasma in litio eparina, per plasma in sodio eparina e plasma in K2 EDTA) rispetto ai campioni di controllo (siero tappo rosso, senza additivo) secondo le linee guida CLSI EP9-A3. È stato valutato un totale di 20 campioni (16 nativi, 4 additivati) per coprire l'intervallo del test. L'analisi di regressione lineare è stata effettuata su dati comparativi:

Tipo di campione	Pendenza [IC 95%]	Intercetta (ng/mL) [IC 95%]	Coefficiente di correlazione (r)
SST	1,02 [da 0,94 a 1,09]	0,19 [da -1,34 a 1,72]	0,99
Litio eparina	0,93 [da 0,82 a 1,04]	0,73 [da -1,49 a 2,95]	0,97
Sodio eparina	0,93 [da 0,84 a 1,01]	0,64 [da -1,00 a 2,28]	0,98
EDTA	0,96 [da 0,86 a 1,05]	0,65 [da -1,19 a 2,49]	0,98

16. LIMITAZIONI D'USO

- Come nel caso di qualsiasi procedura diagnostica, i risultati devono essere interpretati unitamente ai dati clinici del paziente e alle altre informazioni a disposizione del medico.
- Non sono state stabilite le caratteristiche di azione di questo dosaggio nella popolazione pediatrica.
- Gli anticorpi eterofili nel siero umano possono reagire con le immunoglobuline dei reagenti, interferendo con gli immunodosaggi *in vitro*[22]. I pazienti regolarmente esposti agli animali o a prodotti derivati da siero animale possono essere soggetti a questa interferenza, quindi si potrebbero osservare valori anomali.
- La presenza di immunocomplessi o altri aggregati di immunoglobuline nel campione del paziente può causare un aumento del livello di legame non specifico e produrre falsi positivi in questo test.

17. GESTIONE DEI RIFIUTI

I reagenti devono essere smaltiti in conformità alle normative locali.

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19. IDENTIFICATORE DELLE REVISIONI

Le aggiunte o le modifiche alle istruzioni per l'uso sono indicate dall'evidenziazione in grigio.

20. RECLAMI SUI PRODOTTI E SUPPORTO TECNICO

Per un paziente/utente/terza parte nell'Unione Europea e nei Paesi con un regime normativo simile (Regulation 2017/746/EU on IVD Medical Devices); se, durante l'uso di questo dispositivo o come risultato del suo utilizzo, si è verificato un incidente grave, segnalarlo al produttore e/o al suo rappresentante autorizzato e all'autorità normativa nazionale.
Il produttore può essere contattato tramite il relativo servizio clienti o il team di supporto tecnico. I dettagli di contatto sono disponibili di seguito e sul sito Web dell'azienda: www.diametra.com.

Ed. 03/2022 **DCM144-2**

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CE

DiaMetra
care for quality

DCM144-2
Ed. 03/2022

Anti Cardiolipin Screen

for routine analysis

Quantitative determination of IgG or IgM autoantibodies against Cardiolipin in human serum or plasma

IVD | LOT See external label | 2°C 8°C | Σ = 96 tests | REF DKO144

1. INTENDED PURPOSE

For *In Vitro* Diagnostic Use
For Laboratory Professional Use

Anti Cardiolipin Screen is a manual *in vitro* diagnostic device intended for the quantitative determination of both IgG and IgM class antibodies directed against cardiolipin in human serum or plasma. Results are to be used in conjunction with other clinical and laboratory data as an aid in the diagnosis of antiphospholipid syndrome (APS).

2. CLINICAL SIGNIFICANCE

Cardiolipin is a negatively charged phospholipid which is typically located in the inner mitochondrial membrane[1]. Autoantibodies directed against cardiolipin are part of a group known as antiphospholipid antibodies which includes anti-β2 glycoprotein 1 autoantibodies. Measurement of anti-cardiolipin autoantibodies is considered to be one of the most important markers to support diagnosis of antiphospholipid syndrome (APS)[2,3].

APS is a systemic autoimmune disorder characterised by a combination of arterial and/or venous thromboses, pregnancy complications, such as recurrent foetal loss, together with elevated levels of antiphospholipid antibodies[4]. APS was first described in patients with systemic lupus erythematosus (SLE), though it has subsequently been established that SLE may be independent of an underlying disease[5].

APS can occur alone – Primary APS, or in association with other conditions, such as SLE – Secondary APS[6] (6). However, it has been demonstrated that anti-cardiolipin antibodies (aCLs) can be detected in patients with SLE that do not develop Secondary APS[7-9]. However, thromboembolic events are the most common clinical manifestation of APS

Anti-cardiolipin antibodies can recognise both cardiolipin and portions of the phospholipid-protein complex β2 glycoprotein 1-cardiolipin[10,11].

Studies have indicated an association of anti-cardiolipin IgG and IgM antibodies[2,7,11-16], with thrombotic events whereas others suggest these to be linked with IgG isotype, but not IgM[6,10,17].

aCL IgM antibodies have been shown to occur in infections such as chronic hepatitis C, leprosy, syphilis, but they are not directly involved in thrombotic events[17].

It is likely that the presence of antiphospholipid antibodies, including aCL IgG and IgM constitutes the single most recognisable risk factor in cases of recurrent pregnancy loss and late placenta-mediated obstetric complications[4-6,11,14-15,18,19]. Where patients can present with only adverse pregnancy outcomes with isolated vascular events or with both obstetric and thrombotic manifestations[6]. It has been suggested that anti-β2-glycoprotein I – cardiolipin antibodies are able to recognise the antigen on placental tissues, inhibiting the growth and differentiation of trophoblasts which may eventually cause defective placentation[20].

3. PRINCIPLE OF THE METHOD

The Anti Cardiolipin Screen allows the determination of autoantibodies directed against the Cardiolipin-β2-glycoprotein complex through two different calibration curves and enzyme conjugates (one specific for IgG test, one specific for IgM test) and one microplate. The principle of the method and assay procedure are the same for both assessments. Use reagents for IgG or reagents for IgM depending on the isotype which is under investigation.

The Anti Cardiolipin Screen is a two-step sandwich enzyme immunometric assay (ELISA) where patient samples, calibrators or controls are incubated on microtitre plates coated with the antigenic cardiolipin-β2 glycoprotein complex. During the incubation, antibodies present in the test sample bind to the immobilised antigen complex. After the incubation, the bound/free separation is performed by a simple solid phase washing.

A subsequent incubation occurs with anti-human IgM or IgG conjugated with horseradish peroxidase (HRP), which binds to the immobilised antibodies. A further wash step is performed to remove excess conjugate. Then, a chromogenic substrate solution containing TMB is dispensed into the wells which reacts with the conjugated HRP and a blue colour develops that changes into yellow when the Stop Solution (H_2SO_4) is added. The colour intensity is directly proportional to the Anti Cardiolipin IgM or IgG concentration (depending on the conjugate used) in the sample.

Anti-cardiolipin antibody concentration in the sample is calculated through a calibration curve.

4. REAGENTS, MATERIALS AND INSTRUMENTATION

4.1. Reagents and materials supplied in the kit

For determination of IgG class antibodies

1. Anti Cardiolipin IgG Calibrators
(5 vials, 1.2 mL each)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

CAL0	**REF DCE002/11306-0**
CAL1	**REF DCE002/11307-0**
CAL2	**REF DCE002/11308-0**
CAL3	**REF DCE002/11309-0**
CAL4	**REF DCE002/11310-0**

2. Controls (2 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

Negative Control	**REF DCE045/11301-0**
Positive Control	**REF DCE045/11302-0**

3. Conjugate IgG (1 vial, 15 mL)
Anti h-IgG conjugate with horseradish peroxidase (HRP), BSA 0.1%, ProClin < 0.0015% **REF DCE002/11302-0**

For determination of IgM class antibodies

1. Calibrators (5 vials, 1.2 mL each)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

CAL0	**REF DCE002/11206-0**
CAL1	**REF DCE002/11207-0**
CAL2	**REF DCE002/11208-0**
CAL3	**REF DCE002/11209-0**
CAL4	**REF DCE002/11210-0**

2. Controls (2 vials, 1.2 mL each, ready to use)
Phosphate buffer 0.1M, NaN_3 < 0.1%, human serum

Negative Control	**REF DCE045/11201-0**
Positive Control	**REF DCE045/11202-0**

3. Conjugate (1 vial, 15 mL)
Anti h-IgM conjugate with horseradish peroxidase (HRP), BSA 0.1%, ProClin < 0.0015% **REF DCE002/11202-0**

Common reagents

4. Sample Diluent (1 vial, 100 mL)
Phosphate buffer 0.1 M NaN_3 < 0.1%
REF DCE053-0

5. Coated Microplate (1 breakable microplate)
Microplate coated with antigenic Cardiolipin-β2-Glycoprotein complex **REF DCE002/14403-0**

6. TMB Substrate (1 vial, 15 mL)
H_2O_2 -TMB (0.26 g/L) *(avoid any skin contact)*
REF DCE004-0

7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(avoid any skin contact)*
REF DCE005-0

8. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0.2M, pH 7.4 **REF DCE054-0**

4.2. Materials required but not provided

Distilled water

4.3. Auxiliary materials and instrumentation

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

5. WARNINGS

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

6. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. Any exceptions are clearly indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- **WARNING: the conjugate reagent is designed to ensure maximum dose sensitivity and may be contaminated by external agents if not used properly;** therefore, it is recommended to use disposable consumables (tips, bottles, trays, etc.). For divided doses, take the exact amount of conjugate needed and do not re-introduce any waste product into the original bottle. In addition, **for doses dispensed with the aid of automatic and semi-automatic devices,** before using the conjugate, it is advisable to clean the fluid handling system, ensuring that the

procedures of washing, deproteinisation and decontamination are effective in avoiding contamination of the conjugate; this procedure is highly recommended when the kit is processed using analysers which are not equipped with disposable tips.
For this purpose, DiaMetra supplies a separate decontamination reagent for cleaning needles.

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

7. REAGENT STORAGE AND STABILITY

Store the kit at 2 – 8°C in the dark.

- The kit is stable at 2 – 8°C until the expiry date stated on the external kit label.
- Once opened, the kit is stable at 2 – 8°C for 6 months.
- The diluted wash solution is stable for 30 days at 2-8°C.

Important note: open the bag containing the Coated Microplate only when it is at room temperature and close it immediately after use.

8. SAMPLE COLLECTION AND STORAGE

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

Sample Storage	Duration
2 – 8 °C	96 hours
Freeze/thaw cycles	3 cycles

9. PROCEDURE

9.1. Preparation of Calibrators and Controls

The calibrators and controls are ready to use.
The calibrators and approximately the following concentrations:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

9.2. Preparation of the Wash Solution

Dilute the content of the vial “10X Conc. Wash Solution” with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio.

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

9.3. Preparation of Samples

All serum and plasma samples must be diluted 1:100 with sample diluent.
e.g., 10 µL of sample should be diluted with 990 µL of sample diluent.

9.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay, immediately store the reagents at 2-8°C: avoiding long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each control, two for each sample, one for blank.

The following procedure is the same for both class IgG and IgM antibodies assay.

Reagents	Calibrator	Sample/ Controls	Blank
Use reagents for IgG or reagents for IgM depending on the isotype which is under investigation			
Calibrator C_0-C_4 (IgG or IgM)	**100 µL**		
Controls (IgG or IgM)		**100 µL**	
Diluted Sample		**100 µL**	
Incubate for 60 minutes at room temperature (22-28°C). Remove the content from each well; wash the wells 3 times with 300 µL diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate (IgG or IgM)	**100 µL**	**100 µL**	
Incubate for 60 minutes at room temperature (22-28°C). Remove the content from each well; wash the wells 3 times with 300 µL diluted wash solution. **Washing**: follow the same indications of the previous point.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate for 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

10. QUALITY CONTROL

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

The kit controls provided in the kit should be tested as unknowns and are intended to assist in assessing the validity of results obtained with each assay plate.

The mean concentration of each control level is documented in the QC report included with each kit. These mean concentration levels are determined over several assays which are run in duplicate in multiple locations across each plate. This test is only valid if the optical density at 450 nm for the controls as well as for the calibrators (C_0-C_4) fall within with the respective range indicated on the Quality Control Certificate enclosed in each test kit.

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

When interpreting control data, users should note that this product was designed and developed as a manual product. The range stated on the QC certificate should be appropriate for assays that are performed manually and with strict adherence to the Assay Procedure described above. It is recognised by Quality Control professionals, that as a result of differences in conditions and practices, there will always be variability in the mean values and precision of control measurements between different laboratories[21].

11. CALCULATION OF RESULTS

A variety of data reduction software packages are available, which may be employed to generate the mean calibration curve and to calculate the mean concentrations of unknown samples and controls. A 4-parameter logistic (4PL) curve fit with lin-log coordinates, **including Calibrator 0 is required.** A smoothed spline fit including Calibrator 0 can be used. Other curve fitting algorithms are not recommended.

Alternatively, a calibration curve may be prepared on semi-log graph paper by plotting mean absorbance on the Y-axis against concentration of analyte on the X-axis. Calibrator 0 should be included in the calibration curve. Read the mean absorbance value of each unknown sample off the curve.

12. MEASURING RANGE

12.1. For assessment of IgG class antibodies

The assay measuring range (AMR) is 2 – 80 AU/mL.
Any value that reads below 2 AU/mL should be reported as "< 2 AU/mL". Any value that reads above 80 AU/mL should be reported as "> 80 AU/mL".

12.2. For assessment of IgM class antibodies

The assay measuring range (AMR) is 2.08 – 80 AU/mL.
Any value that reads below 2.09 AU/mL should be reported as "< 2.08 AU/mL". Any value that reads above 80 AU/mL should be reported as "> 80 AU/mL".

13. METROLOGY AND TRACEABILITY

13.1. For assessment of IgG class antibodies

The calibrators of this kit are traceable to the Centres for Disease Control (CDC) Human IgG Anti-Cardiolipin Monoclonal Antibody HCAL - Catalogue [IS2717].

13.2. For assessment of IgM class antibodies

The calibrators of this kit are traceable to the Centres for Disease Control (CDC) Human IgM Anti-Cardiolipin Monoclonal Antibody EY2C9 [IS2718].

14. INTERPRETATION OF RESULTS

Concentration	Interpretation
< 8 AU/mL	The sample should be considered negative
8 – 10 AU/mL	The sample should be graded equivocal and repeat testing / sampling should be performed according to internal practices
>10 AU/mL	The sample should be considered positive

Determination of a range of expected values for a "normal" population of a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore, each laboratory should consider the range given by the Manufacturer as a general indication and produce their own range of expected values based on the indigenous population.
Positive results should be verified concerning the entire clinical status of the patient, with the decision for therapy being taken on an individual basis. It is recommended that each laboratory establishes its own normal and pathological ranges of Anti-Cardiolipin antibody values.

15. PERFORMANCE CHARACTERISTICS

Representative performance data are shown. Results obtained at individual laboratories may vary.

15.1. For assessment of IgG class antibodies

15.1.1. Detection Capability

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

Sensitivity	Concentration
Limit of Blank (LoB)	0.59 AU/mL
Limit of Detection (LoD)	1.25 AU/mL
Limit of Quantitation (LoQ)	2.00 AU/mL

15.1.2. Trueness

Trueness of the Anti Cardiolipin Screen for assessment of IgG class antibodies has been demonstrated through performance of a recovery test using the CDC Human IgG Anti-Cardiolipin Monoclonal Antibody HCAL - Catalogue [IS2717].

15.1.3. Diagnostic Sensitivity and Specificity

The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" using 50 negative and 51 positive samples run on two reagent lots.

		DKO144 - IgG		Total
		Positive	Negative	
True state	Positive	47	4	**51**
	Negative	0	57	**57**
Total		**47**	**61**	**108**

Diagnostic sensitivity: 92%

Diagnostic specificity: 100%

15.1.4. Precision

Precision of the Anti Cardiolipin Screen for determination of IgG class antibodies was determined by performing a complex precision study.

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

Sample	n	Mean Conc. (AU/mL)	Within run (Repeatability) SD	CV
1	75	6.95	0.38	5.5%
2	75	11.03	0.51	4.6%
3	75	20.12	0.94	4.7%
4	75	30.26	1.86	6.1%
5	75	50.11	2.58	5.1%
6	75	71.71	2.53	3.5%

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

Sample	n	Mean Conc. (AU/mL)	Within Laboratory (Reproducibility) SD	CV%
1	150	6.98	0.49	7.0%
2	150	11.17	0.84	7.5%
3	150	20.16	1.87	9.3%
4	150	30.38	3.06	10.1%
5	150	50.76	5.02	9.9%
6	150	72.30	3.93	5.4%

15.1.5. Linearity

Linearity was evaluated based on CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". For anti-cardiolipin IgG concentration by Anti Cardiolipin Screen, the measurement procedure shows linearity for the interval from 0.84 to 83.68 ng/mL within the allowable deviation of linearity (ADL) of ±15 %.

15.2. Analytical Specificity

The following substances do not interfere with a bias of > ±15% in the Anti Cardiolipin Screen assay when assessing IgG class antibodies when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin, conjugated	15 mg/dL
Bilirubin, unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglyceride	500 mg/dL

15.2.1. Serum-plasma study

The Anti Cardiolipin Screen matrix comparison study for assessment of IgG class antibodies was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP9-A3 guidelines. A total of 22 samples (18 native, 4 spiked) to cover the range were evaluated. Linear regression analysis was performed on the comparative data:

Sample type	Slope [95% CI]	Intercept (ng/mL) [95% CI]	Correlation coefficient (r)
SST	0.96 [0.92 – 0.98]	0.64 [-0.38 to 1.66]	1.00
Lithium Heparin	0.92 [0.88 to 0.96]	0.87 [-0.24 to 1.99]	1.00
Sodium Heparin	0.94 [0.89 to 0.98]	0.66 [-0.75 to 2.06]	0.99
EDTA	0.95 [0.92 to 0.99]	0.54 [-0.69 to 1.77]	1.00

15.3. For assessment of IgM class antibodies

15.3.1. Detection Capability

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

Sensitivity	Concentration
Limit of Blank (LoB)	0.76 AU/mL
Limit of Detection (LoD)	1.45 AU/mL
Limit of Quantitation (LoQ)	2.08 AU/mL

15.3.2. Trueness

Trueness of the Anti Cardiolipin Screen for assessment of IgM class antibodies demonstrated through performance of a recovery test using the CDC Human IgM Anti-Cardiolipin Monoclonal Antibody EY2C9 [IS2718].

15.3.3. Diagnostic Sensitivity and Specificity

The sensitivity and specificity were determined with guidance from CLSI EP-24 "Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves" using 73 negative and 62 positive samples run on two reagent lots.

		DKO144 IgM		Total
		Positive	Negative	
True state	Positive	50	12	**62**
	Negative	0	73	**73**
Total		**50**	**85**	**135**

Diagnostic sensitivity: 80%

Diagnostic specificity: 100%

15.3.4. Precision

Precision of the Anti Cardiolipin Screen for determination of IgM class antibodies determined by performing a complex precision study.

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

Sample	n	Mean Conc. (AU/mL)	Within run (Repeatability) SD	CV
1	75	7.74	0.40	5.2%
2	75	12.46	0.51	4.1%
3	75	21.06	0.99	4.7%
4	75	32.07	1.46	4.6%
5	75	55.15	1.19	2.2%
6	75	75.45	2.33	3.1%

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

Sample	n	Mean Conc. (AU/mL)	Within Laboratory (Reproducibility)	
			SD	CV%
1	150	7.65	0.46	6.0%
2	150	12.33	0.74	6.0%
3	150	21.03	1.60	7.6%
4	150	31.89	1.98	6.2%
5	150	54.28	2.70	5.0%
6	150	75.46	2.63	3.5%

15.3.5. Linearity

Linearity was evaluated based on CLSI EP-06, "Evaluation of the Linearity of Quantitative Measurement Procedures". For anti-cardiolipin IgM concentration by Anti Cardiolipin Screen, the measurement procedure shows linearity for the interval from 0.82 to 86.88 AU/mL within the allowable deviation of linearity (ADL) of ±15 %.

15.3.6. Analytical Specificity

The following substances do not interfere with a bias of > ±15% in the Anti Cardiolipin Screen assay when assessing IgM class antibodies when the concentrations are below the stated threshold presented in the following table.

Potentially Interfering Reagent	Threshold Concentration
Bilirubin, conjugated	15 mg/dL
Bilirubin, unconjugated	15 mg/dL
Haemoglobin	200 mg/dL
Total Protein	10 g/dL
Triglyceride	500 mg/dL

15.3.7. Serum-plasma study

The Anti Cardiolipin Screen matrix comparison study for assessment of IgM class antibodies was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K2 EDTA plasma) versus the control samples (red top serum, without additive) following CLSI EP9-A3 guidelines. A total of 20 samples (16 native, 4 spiked) to cover the assay range were evaluated. Linear regression analysis was performed on the comparative data:

Sample type	Slope [95% CI]	Intercept (ng/mL) [95% CI]	Correlation coefficient (r)
SST	1.02 [0.94 to 1.09]	0.19 [-1.34 to 1.72]	0.99
Lithium Heparin	0.93 [0.82 to 1.04]	0.73 [-1.49 to 2.95]	0.97
Sodium Heparin	0.93 [0.84 to 1.01]	0.64 [-1.00 to 2.28]	0.98
EDTA	0.96 [0.86 to 1.05]	0.65 [-1.19 to 2.49]	0.98

16. LIMITATIONS OF USE

- As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
- The performance characteristics of this assay have not been established in a paediatric population.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays[22]. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
- The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.

17. WASTE MANAGEMENT

Reagents must be disposed of in accordance with local regulations.

18. BIBLIOGRAPHY

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19. REVISION IDENTIFIER

Additions or changes to the IFU are indicated by grey highlighting.

20. PRODUCT COMPLAINTS AND TECHNICAL SUPPORT

For a patient/user/third party in the European Union and in countries with similar regulatory regime (Regulation 2017/746/EU on IVD Medical Devices); if, during the use of this device or as a result of its use, a serious incident has occurred, please report it to the manufacturer and/or its authorised representative and to your national regulatory authority.
The manufacturer can be contacted through their customer service or technical support team. The contact details can be found below and on the company website: www.diametra.com.

Ed. 03/2022 **DCM144-2**

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CE

DiaMetra
care for quality

DCM144-2
Ed. 03/2022

Anti Cardiolipin Screen

para el análisis de rutina

Determinación cuantitativa de autoanticuerpos IgG o IgM contra la cardiolipina en suero o plasma humano

IVD | **LOT** Ver etiqueta externa | 2°C 8°C | Σ = 96 pruebas | **REF DKO144**

1. FINALIDAD PREVISTA

Para uso en diagnóstico *in vitro*
Para uso profesional de laboratorio

Anti Cardiolipin Screen es un dispositivo manual de diagnóstico *in vitro* destinado a la determinación cuantitativa de anticuerpos tanto IgG como IgM dirigidos contra la cardiolipina en suero o plasma humano. Los resultados deben utilizarse como ayuda en el diagnóstico del síndrome antifosfolípido (SAF) junto con otros datos clínicos y de laboratorio.

2. IMPORTANCIA CLÍNICA

La cardiolipina es un fosfolípido con carga negativa que suele estar localizado en la membrana mitocondrial interna[1]. Los autoanticuerpos dirigidos contra la cardiolipina forman parte de un grupo conocido como anticuerpos antifosfolípidos que incluye los autoanticuerpos anti-β2 glicoproteína 1. La medición de los autoanticuerpos anticardiolipina se considera uno de los marcadores más importantes para apoyar el diagnóstico del síndrome antifosfolípido (SAF)[2,3].

El SAF es un trastorno autoinmunitario sistémico caracterizado por una combinación de trombos arteriales y/o venosos, complicaciones del embarazo, como la pérdida gestacional recurrente, y niveles elevados de anticuerpos antifosfolípidos[4]. El SAF se describió por primera vez en pacientes con lupus eritematoso sistémico (LES), aunque posteriormente se ha establecido que el LES puede existir de forma independiente a una enfermedad subyacente[5].

El SAF puede darse por sí mismo (SAF primario) o puede estar asociado a otras condiciones médicas como el LES (SAF secundario)[6] (6). Sin embargo, se ha demostrado que pueden detectarse anticuerpos anticardiolipina (aCL) en pacientes con LES que no desarrollan SAF secundario[7-9]. No obstante, los eventos tromboembólicos son la manifestación clínica más común del SAF.

Los anticuerpos anticardiolipina pueden reconocer tanto la cardiolipina como partes del complejo de fosfolípidos y proteínas β2 glicoproteína 1-cardiolipina[10,11].

Existen estudios que indican una asociación de los anticuerpos anticardiolipina IgG e IgM[2,7,11-16] con los eventos trombóticos, mientras que otros sugieren que estos están relacionados con el isotipo IgG, pero no con el IgM[6,10,17].

Se ha demostrado que los anticuerpos IgM aCL aparecen en infecciones como la hepatitis C crónica, la lepra o la sífilis, pero no están directamente implicados en eventos trombóticos[17].

Es probable que la presencia de anticuerpos antifosfolípidos, incluidos los aCL IgG e IgM, constituya el factor de riesgo más reconocible en los casos de pérdida de recurrente del embarazo y de complicaciones obstétricas mediadas por la placenta tardía[4-6,11,14-15,18,19]. Las pacientes pueden presentar únicamente resultados adversos del embarazo con eventos vasculares aislados o con manifestaciones tanto obstétricas como trombóticas[6]. Se ha sugerido que los anticuerpos anti-β2-glicoproteína 1-cardiolipina son capaces de reconocer el antígeno en los tejidos de la placenta, inhibiendo el crecimiento y la diferenciación de los trofoblastos, lo que puede causar finalmente una placentación defectuosa[20].

3. PRINCIPIO DEL MÉTODO

Anti Cardiolipin Screen permite la determinación de autoanticuerpos dirigidos contra el complejo cardiolipina-β2-glicoproteína mediante dos curvas de calibración y conjugados enzimáticos diferentes (uno específico para la prueba IgG y otro específico para la prueba IgM) y una microplaca. El principio del método y el procedimiento de ensayo son los mismos para ambas evaluaciones. Utilice reactivos para IgG o reactivos para IgM según el isotipo que se esté investigando.

El ensayo Anti Cardiolipin Screen es un ensayo enzimático inmunométrico (ELISA) de dos pasos tipo sándwich en el que las muestras de los pacientes, los calibradores o los controles se incuban en placas de microtitulación recubiertas con el complejo antigénico cardiolipina-β2 glicoproteína. Durante la incubación, los anticuerpos presentes en la muestra de ensayo se unen al complejo de antígenos inmovilizados. Tras la incubación, la separación ligada/libre se realiza mediante un simple lavado en fase sólida.

A continuación, se realiza una incubación con IgM o IgG antihumano conjugado con peroxidasa de rábano picante (HRP), que se une a los anticuerpos inmovilizados. Se realiza otro paso de lavado para eliminar el exceso de conjugado. A continuación, se dispensa en los pocillos una solución de sustrato cromogénico que contiene TMB, que reacciona con el HRP conjugado y se desarrolla un color azul que cambia a amarillo cuando se añade la solución de detención (H_2SO_4).

La intensidad del color es directamente proporcional a la concentración de anticardiolipina IgM o IgG (dependiendo del conjugado que se utilice) de la muestra.

La concentración de anticuerpos anticardiolipina en la muestra se calcula mediante una curva de calibración.

4. REACTIVOS, MATERIALES E INSTRUMENTACIÓN

4.1. Reactivos y materiales incluidos en el kit

Para la determinación de anticuerpos de clase IgG

1. Calibradores de anticardiolipina IgG
(5 viales de 1,2 mL cada uno)
Tampón fosfato 0,1 M, NaN_3 < 0,1 %, suero humano

CAL0	**REF DCE002/11306-0**
CAL1	**REF DCE002/11307-0**
CAL2	**REF DCE002/11308-0**
CAL3	**REF DCE002/11309-0**
CAL4	**REF DCE002/11310-0**

2. Controles (2 viales de 1,2 mL cada uno, listos para usar)
Tampón fosfato 0,1 M, NaN_3 <0,1 %, suero humano

Control negativo	**REF DCE045/11301-0**
Control positivo	**REF DCE045/11302-0**

3. Conjugado IgG (1 vial, 15 mL)
IgG antihumano conjugado con peroxidasa de rábano picante (HRP), BSA 0,1 %, ProClin < 0,0015 %
REF DCE002/11302-0

Para la determinación de anticuerpos de clase IgM

1. Calibradores (5 viales de 1,2 mL cada uno)
Tampón fosfato 0,1 M, NaN_3 < 0,1 %, suero humano

CAL0	**REF DCE002/11206-0**
CAL1	**REF DCE002/11207-0**
CAL2	**REF DCE002/11208-0**
CAL3	**REF DCE002/11209-0**
CAL4	**REF DCE002/11210-0**

2. Controles (2 viales de 1,2 mL cada uno, listos para usar)
Tampón fosfato 0,1 M, NaN_3 <0,1 %, suero humano

Control negativo	**REF DCE045/11201-0**
Control positivo	**REF DCE045/11202-0**

3. Conjugado (1 vial, 15 mL)
IgM antihumano conjugado con peroxidasa de rábano picante (HRP), BSA 0,1 %, ProClin < 0,0015 %
REF DCE002/11202-0

Reactivos comunes

4. Diluyente de muestras (1 vial, 100 mL)
Tampón fosfato 0,1 M, NaN_3 < 0,1 %
REF DCE053-0

5. Microplaca recubierta (1 microplaca que se puede romper)
Microplaca recubierta con el complejo antigénico cardiolipina-β2 glicoproteína **REF DCE002/14403-0**

6. Sustrato de TMB (1 vial, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(evitar el contacto con la piel)*
REF DCE004-0

7. Solución de detención (1 vial, 15 mL)
Ácido sulfúrico 0,15 M *(evitar el contacto con la piel)*
REF DCE005-0

8. Conc. 10X Solución de lavado (1 vial, 50 mL)
Tampón fosfato 0,2 M, pH 7,4 **REF DCE054-0**

4.2. Materiales necesarios pero no suministrados

Agua destilada

4.3. Materiales auxiliares e instrumentación

Dispensador automático
Dispositivos de pipetas de precisión
Lector de microplacas (450 nm, 620-630 nm)

5. ADVERTENCIAS

- Este kit está destinado al uso *in vitro* realizado exclusivamente por profesionales. No es para uso interno o externo en personas ni animales.
- Utilice el equipo de protección personal adecuado cuando trabaje con los reactivos suministrados.
- Siga las prácticas de laboratorio recomendadas (BPL) para manipular productos sanguíneos.
- Todo el material de origen humano utilizado en la preparación de los reactivos ha sido sometido a pruebas que han dado resultado negativo para los anticuerpos contra el VIH-1 y VIH-2, el HbsAg y el VHC. Sin embargo, ningún método de prueba puede ofrecer una garantía total de ausencia de VIH, VHB, VHC u otros agentes infecciosos. Por lo tanto, los calibradores y los controles deben manejarse de la misma manera que el material potencialmente infeccioso.
- El material de origen animal utilizado en la preparación del kit se ha obtenido de animales certificados como sanos y la proteína bovina se ha obtenido de países donde no hay infección de EEB, pero estos materiales deben manejarse como potencialmente infecciosos.
- Algunos reactivos contienen pequeñas cantidades de azida sódica (NaN_3) o ProClin™ 300 como conservante. Evite el contacto con la piel o las mucosas.
- La azida sódica puede ser tóxica si se ingiere o se absorbe a través de la piel o los ojos; además, puede reaccionar con las tuberías de plomo o cobre para formar azidas metálicas potencialmente explosivas. Si elimina los reactivos en un fregadero, lávelos con gran cantidad de agua para evitar la acumulación de azida.
- El sustrato de TMB contiene un irritante que es perjudicial si se inhala, se ingiere o se absorbe a través de la piel. Para evitar lesiones, evite la inhalación, la ingestión o el contacto con la piel y los ojos.
- La solución de detención consiste en una solución diluida de ácido sulfúrico. El ácido sulfúrico es venenoso, corrosivo y puede ser tóxico si se ingiere. Para evitar quemaduras químicas, evite el contacto con la piel y los ojos.
- Evite la exposición del reactivo TMB/H_2O_2 a la luz solar directa, a metales o a oxidantes. No congele la solución.

6. PRECAUCIONES

- Siga estrictamente la secuencia de pasos de pipeteado que se indica en este protocolo. Los datos de rendimiento representados en este documento se obtuvieron utilizando los reactivos específicos indicados en estas instrucciones de uso.
- Todos los reactivos deben conservarse refrigerados entre 2 y 8 °C en su envase original. Las excepciones se indican claramente.
- Deje que todos los componentes del kit y las muestras alcancen la temperatura ambiente (22-28 °C) y mezcle bien antes de usarlos.

- No intercambie componentes del kit procedentes de diferentes lotes. Debe respetarse la fecha de caducidad impresa en las etiquetas de la caja y de los viales. No utilice ningún componente del kit después de su fecha de caducidad.
- **ADVERTENCIA: el reactivo conjugado está diseñado para garantizar la máxima sensibilidad de la dosis y puede contaminarse con agentes externos si no se utiliza correctamente;** por lo tanto, se recomienda utilizar consumibles desechables (puntas, frascos, bandejas, etc.). Para dosis divididas, tome la cantidad exacta de conjugado necesaria y no vuelva a introducir ningún producto de desecho en el frasco original. Además, **para las dosis dispensadas mediante dispositivos automáticos y semiautomáticos,** antes de utilizar el conjugado, es aconsejable limpiar el sistema de manipulación de fluidos, asegurándose de que los procedimientos de lavado, desproteinización y descontaminación sean eficaces para evitar la contaminación del conjugado; este procedimiento es muy recomendable cuando el kit se procesa con analizadores que no están equipados con puntas desechables.
  Para ello, DiaMetra proporciona un reactivo de descontaminación independiente para la limpieza de las agujas.
- Si el usuario utiliza un equipo automatizado, tiene la responsabilidad de asegurarse de que el kit ha sido debidamente probado.
- La eliminación incompleta o imprecisa del líquido de los pocillos podría alterar la precisión del ensayo y/o aumentar el fondo. Para mejorar el rendimiento del kit en sistemas automáticos se recomienda aumentar el número de lavados.
- Es importante que el tiempo de reacción en cada pocillo se mantenga constante para obtener resultados reproducibles. El pipeteo de las muestras no debe prolongarse más de diez minutos para evitar errores en el ensayo. Si se necesitan más de 10 minutos, siga el mismo orden de dispensación. Si se utiliza más de una placa, se recomienda repetir la curva dosis-respuesta en cada placa.
- La adición de la solución de sustrato de TMB inicia una reacción cinética, que finaliza al añadir la solución de detención. Por lo tanto, el sustrato de TMB y la solución de detención deben añadirse en la misma secuencia para eliminar las posibles desviaciones temporales durante la reacción.
- Respete las directrices para realizar el control de calidad en los laboratorios médicos mediante el ensayo de controles y/o sueros combinados.
- Se requiere la máxima precisión en la reconstitución y dispensación de los reactivos.
- No se deben usar en el ensayo muestras contaminadas microbiológicamente, muy lipémicas o hemolizadas.
- Los lectores de placas miden en vertical. No toque el fondo de los pocillos.

7. ALMACENAMIENTO Y ESTABILIDAD DE LOS REACTIVOS

Almacene el kit a 2-8 ºC en un lugar oscuro.

- El kit es estable a 2-8 ºC hasta la fecha de caducidad indicada en su etiqueta externa.
- Una vez abierto, el kit es estable a 2-8 °C durante 6 meses.
- La solución de lavado diluida es estable durante 30 días a 2-8 °C.

Nota importante: abra la bolsa que contiene la microplaca recubierta solo cuando esté a temperatura ambiente y ciérrela inmediatamente después de su uso.

8. RECOGIDA Y ALMACENAMIENTO DE LAS MUESTRAS

El ensayo debe realizarse usando muestras de suero (tubos de muestras estándar o tubos que contienen gel de separación de suero) o plasma (heparina de litio, heparina de sodio o EDTA de potasio).

Almacenamiento de muestras	Duración
2-8 ºC	96 horas
Ciclos de congelación/descongelación	3 ciclos

9. PROCEDIMIENTO

9.1. Preparación de calibradores y controles

Los calibradores y controles están listos para usarse.
Los calibradores tienen aproximadamente las siguientes concentraciones:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

9.2. Preparación de la solución de lavado

Diluir el contenido del vial «10X Conc. Wash Solution» con agua destilada hasta un volumen final de 500 mL antes de usarlo. Para volúmenes más pequeños, respete la relación de dilución de 1:10.

Es posible que observe la presencia de cristales dentro de la solución de lavado concentrada; en este caso, mezcle a temperatura ambiente hasta la completa disolución de los cristales. Para una mayor precisión, diluya todo el frasco de solución de lavado concentrada a 500 mL, teniendo cuidado también de transferir los cristales enjuagando completamente el frasco y luego mezclando hasta que los cristales se disuelvan completamente.

9.3. Preparación de las muestras

Todas las muestras de suero y plasma deben diluirse a una concentración de 1:100 con diluyente de muestras.
Por ejemplo, 10 µL de muestra deben diluirse con 990 µL de diluyente de muestras.

9.4. Procedimiento

- **Deje que todos los reactivos alcancen la temperatura ambiente (22-28 ºC) durante al menos 30 minutos.** Al finalizar el ensayo, almacene inmediatamente los reactivos a 2-8 ºC: evite la exposición prolongada a la temperatura ambiente.
- Las tiras de micropocillos recubiertas no utilizadas deben dejarse de forma segura en el envoltorio de papel de aluminio que contiene desecante y almacenarse a 2-8 ºC.
- Para evitar que se produzca una posible contaminación microbiana y/o química, los reactivos no utilizados nunca se deberán transferir a los viales originales.

- Como es necesario realizar la determinación por duplicado para mejorar la precisión de los resultados de la prueba, prepare dos pocillos para cada punto de la curva de calibración (C_0-C_4), dos para cada control, dos para cada muestra y uno para el blanco.

El siguiente procedimiento es el mismo para el ensayo de anticuerpos de clase IgG e IgM.

Reactivos	Calibrador	Muestra/ Controles	Blanco
Utilice reactivos para IgG o reactivos para IgM según el isotipo que se esté investigando.			
Calibrador C_0-C_4 (IgG o IgM)	**100 µL**		
Controles (IgG o IgM)		**100 µL**	
Muestra diluida		**100 µL**	
Incube durante 60 minutos a temperatura ambiente (22-28 °C). Retire el contenido de cada pocillo, lave los pocillos 3 veces con 300 µL de solución de lavado diluida. **Nota importante**: en cada paso de lavado, agite ligeramente la placa durante 5 segundos y elimine el exceso de solución golpeando la placa invertida sobre un paño de papel absorbente. **Lavadora automática**: si utiliza un equipo automático, lave los pocillos al menos 5 veces.			
Conjugado (IgG o IgM)	**100 µL**	**100 µL**	
Incube durante 60 minutos a temperatura ambiente (22-28 °C). Retire el contenido de cada pocillo, lave los pocillos 3 veces con 300 µL de solución de lavado diluida. **Lavado**: siga las mismas indicaciones del punto anterior.			
Sustrato de TMB	**100 µL**	**100 µL**	**100 µL**
Incube durante 15 minutos en un lugar oscuro a temperatura ambiente (22-28 °C).			
Solución de detención	**100 µL**	**100 µL**	**100 µL**
Agite suavemente la microplaca. Compare la absorbancia (E) a 450 nm con la obtenida con una longitud de onda de referencia de 620-630 nm o con el blanco en un plazo de 5 minutos.			

10. CONTROL DE CALIDAD

Las prácticas de laboratorio recomendadas (BPL) requieren el uso de muestras de control de calidad en cada serie de ensayos para comprobar el rendimiento del ensayo. Los controles deberán tratarse como muestras desconocidas y los resultados deberán analizarse con métodos estadísticos adecuados.

Los controles incluidos en el kit deberán ser probados como desconocidos y están destinados a ayudar a evaluar la validez de los resultados obtenidos con cada placa de ensayo.

La concentración media de cada nivel de control se documenta en el informe de control de calidad que se incluye en cada kit. Los niveles de concentración media se determinan respecto de varios análisis, los cuales se realizan por duplicado en varios puntos diferentes de cada placa. Esta prueba solo es válida si la densidad óptica a 450 nm de los controles y de los calibradores (C_0-C_4) está dentro del rango respectivo indicado en el Certificado de control de calidad que se incluye con cada kit de prueba.

DiaMetra recomienda que los usuarios mantengan registros gráficos de los valores de control que se generan con cada ensayo, incluida la media de ejecución, la DE (desviación estándar) y el % CV. Esta información facilitará los ensayos de tendencia de los controles relacionados con el rendimiento de lotes de control actuales e históricos relativos a los datos de control de calidad proporcionados. La tendencia facilitará la identificación de los análisis que generan valores de control significativamente distintos de su intervalo medio.

Al interpretar los datos de control, los usuarios deberán tener en cuenta que este producto fue diseñado y desarrollado como un producto manual. El rango establecido en el certificado de control de calidad deberá ser adecuado para los ensayos que se realizan manualmente y en estricto cumplimiento del procedimiento de ensayo anteriormente descrito. Los profesionales del control de la calidad reconocen que, como resultado de las diferencias en las condiciones y en las prácticas, siempre habrá variaciones entre laboratorios en los valores medios y en la precisión de las mediciones de control[21].

11. CÁLCULO DE LOS RESULTADOS

Hay disponibles diversos paquetes de software de reducción de datos que se pueden utilizar para generar el promedio de la curva de calibración y para calcular el promedio de las concentraciones de muestras y controles desconocidos. Es necesario un ajuste de curva logístico de 4 parámetros (4PL) con coordenadas lineales y logarítmicas, **incluido el calibrador 0.** También se puede usar un ajuste de splines suavizado que incluya el calibrador 0. No se recomiendan otros algoritmos de ajuste de curva.

También se puede preparar una curva de calibración en papel semilogarítmico mediante el trazado de la absorbancia media en el eje Y frente a la concentración de analitos en el eje X. El calibrador 0 debe incluirse en la curva de calibración. Lea el valor de absorbancia medio de cada muestra desconocida que se encuentra fuera de la curva.

12. RANGO DE MEDICIÓN

12.1. Para la evaluación de anticuerpos de clase IgG

El rango de medición del ensayo (AMR) es de 2-80 AU/mL. Cualquier valor que sea inferior a 2 AU/mL debe informarse como «< 2 AU/mL». Cualquier valor que sea superior a 80 AU/mL debe informarse como «> 80 AU/mL».

12.2. Para la evaluación de anticuerpos de clase IgM
El rango de medición del ensayo (AMR) es de 2,08-80 AU/mL.
Cualquier valor que sea inferior a 2,09 AU/mL debe informarse como «< 2,08 AU/mL». Cualquier valor que sea superior a 80 AU/mL debe informarse como «> 80 AU/mL».

13. METROLOGÍA Y TRAZABILIDAD

13.1. Para la evaluación de anticuerpos de clase IgG
Los calibradores de este kit son trazables según el catálogo del Centro para el Control de Enfermedades (CDC) sobre el anticuerpo humano monoclonal anticardiolipina IgG HCAL [IS2717].

13.2. Para la evaluación de anticuerpos de clase IgM
Los calibradores de este kit son trazables según el estándar EY2C9 del Centro para el Control de Enfermedades (CDC) sobre el anticuerpo humano monoclonal anticardiolipina IgM [IS2718].

14. INTERPRETACIÓN DE LOS RESULTADOS

Concentración	Interpretación
<8 AU/mL	La muestra debe considerarse negativa.
8-10 AU/mL	La muestra debe ser calificada como equívoca y la repetición de la prueba/muestreo debe realizarse de acuerdo con las prácticas internas.
> 10 AU/mL	La muestra debe considerarse positiva.

La determinación de un rango de valores esperados para una población «normal» de un método dado depende de muchos factores, como la especificidad y la sensibilidad del método utilizado y el tipo de población en investigación. Por lo tanto, cada laboratorio debe considerar el rango dado por el fabricante como una indicación general y establecer su propio rango de valores esperados en función de la población autóctona.

Los resultados positivos deben verificarse en relación con el estado clínico general del paciente, y la decisión de la terapia se toma de forma individual. Se recomienda que cada laboratorio establezca sus propios rangos normales y patológicos de valores de anticuerpos anticardiolipina.

15. CARACTERÍSTICAS DE RENDIMIENTO

Se muestran los datos de rendimiento representativos. Los resultados obtenidos en diferentes laboratorios pueden diferir.

15.1. Para la evaluación de anticuerpos de clase IgG

15.1.1. Capacidad de detección
El límite de blanco (LoB), el límite de detección (LoD) y el límite de cuantificación (LoQ) se determinaron con orientación del documento CLSI EP17-A, “Protocols for Determination of Limits of Detection and Limits of Quantitation”, usando 6 blancos y 6 muestras de bajo nivel.

Sensibilidad	Concentración
Límite de blanco (LoB)	0,59 AU/mL
Límite de detección (LoD)	1,25 AU/mL
Límite de cuantificación (LoQ)	2,00 AU/mL

15.1.2. Veracidad
La veracidad del ensayo Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgG se ha demostrado mediante la realización de una prueba de recuperación utilizando el catálogo del CDC sobre el anticuerpo humano monoclonal anticardiolipina IgG HCAL [IS2717].

15.1.3. Sensibilidad y especificidad del diagnóstico
La sensibilidad y la especificidad se determinaron con orientación del documento CLSI EP-24 “Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves”, usando 50 muestras negativas y 51 positivas realizadas en dos lotes de reactivos.

		DKO144 - IgG		Total
		Positivo	Negativo	
Estado real	Positivo	47	4	51
	Negativo	0	57	57
Total		47	61	108

Sensibilidad del diagnóstico: 92 %

Especificidad del diagnóstico: 100 %

15.1.4. Precisión
La precisión del ensayo Anti Cardiolipin Screen para la determinación de anticuerpos de clase IgG se determinó mediante la realización de un estudio de precisión complejo.

Repetibilidad: Se analizaron un total de 6 muestras de suero en 5 réplicas, una vez al día durante 5 días por 3 operadores.
A continuación se muestran los datos de un lote representativo:

Muestra	n	Medio conc. (AU/mL)	Intraprueba (repetibilidad)	
			DE	CV
1	75	6,95	0,38	5,5 %
2	75	11,03	0,51	4,6 %
3	75	20,12	0,94	4,7 %
4	75	30,26	1,86	6,1 %
5	75	50,11	2,58	5,1 %
6	75	71,71	2,53	3,5 %

Reproducibilidad: Se analizaron un total de 6 muestras de suero en 5 réplicas, una vez al día durante 5 días por 3 operadores.
A continuación se muestran los resultados de los datos combinados de dos lotes:

Muestra	n	Medio conc. (AU/mL)	Dentro del laboratorio (reproducibilidad)	
			DE	CV %
1	150	6,98	0,49	7,0 %
2	150	11,17	0,84	7,5 %
3	150	20,16	1,87	9,3 %
4	150	30,38	3,06	10,1 %
5	150	50,76	5,02	9,9 %
6	150	72,30	3,93	5,4 %

15.1.5. Linealidad
La linealidad se evaluó en base a CLSI EP-06, “Evaluation of the Linearity of Quantitative Measurement Procedures”. Para la concentración de anticardiolipina IgG mediante el ensayo Anti Cardiolipin Screen, el procedimiento de medición muestra linealidad para el intervalo de 0,84 a 83,68 ng/mL dentro de la desviación de linealidad permitida (ADL) de ±15 %.

15.2. Especificidad analítica
Las siguientes sustancias no interfieren con un sesgo de > ±15 % en el ensayo Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgG cuando las concentraciones están por debajo del umbral indicado presentado en la siguiente tabla.

Reactivos que pueden interferir	Límite máximo de concentración
Bilirrubina, conjugada	15 mg/dL
Bilirrubina, no conjugada	15 mg/dL
Hemoglobina	200 mg/dL
Proteína total	10 g/dL
Triglicéridos	500 mg/dL

15.2.1. Estudio en suero-plasma
El estudio de comparación de la matriz de Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgG se realizó para evaluar la diferencia entre los tipos de tubos (tubos separadores de suero [SST], plasma de heparina de litio, plasma de heparina sódica y plasma K2 EDTA) frente a las muestras de control (tapón rojo para suero, sin aditivos) siguiendo las directrices de CLSI EP9-A3. Se evaluó un total de 22 muestras (18 nativas, 4 con aditivos) para cubrir el intervalo. Se realizó un análisis de regresión lineal sobre los datos comparativos:

Tipo de muestra	Pendiente [IC del 95 %]	Intersección (ng/mL) [IC del 95 %]	Coeficiente de correlación (r)
SST	0,96 [0,92 a 0,98]	0,64 [–0,38 a 1,66]	1,00
Heparina de litio	0,92 [0,88 a 0,96]	0,87 [–0,24 a 1,99]	1,00
Heparina sódica	0,94 [0,89 a 0,98]	0,66 [–0,75 a 2,06]	0,99
EDTA	0,95 [0,92 a 0,99]	0,54 [–0,69 a 1,77]	1,00

15.3. Para la evaluación de anticuerpos de clase IgM

15.3.1. Capacidad de detección
El límite de blanco (LoB), el límite de detección (LoD) y el límite de cuantificación (LoQ) se determinaron con orientación del documento CLSI EP17-A, “Protocols for Determination of Limits of Detection and Limits of Quantitation”, usando 6 blancos y 6 muestras de bajo nivel.

Sensibilidad	Concentración
Límite de blanco (LoB)	0,76 AU/mL
Límite de detección (LoD)	1,45 AU/mL
Límite de cuantificación (LoQ)	2,08 AU/mL

15.3.2. Veracidad
La veracidad del ensayo Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgM se ha demostrado mediante la realización de una prueba de recuperación utilizando el estándar EY2C9 del CDC sobre el anticuerpo humano monoclonal anticardiolipina IgM [IS2718].

15.3.3. Sensibilidad y especificidad del diagnóstico
La sensibilidad y la especificidad se determinaron con orientación del documento CLSI EP-24 “Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves”, usando 73 muestras negativas y 62 positivas realizadas en dos lotes de reactivos.

		DKO144 IgM		Total
		Positivo	Negativo	
Estado real	Positivo	50	12	62
	Negativo	0	73	73
Total		50	85	135

Sensibilidad del diagnóstico: 80 %

Especificidad del diagnóstico: 100 %

15.3.4. Precisión

La precisión del ensayo Anti Cardiolipin Screen para la determinación de anticuerpos de clase IgM se determinó mediante la realización de un estudio de precisión complejo.

Repetibilidad: Se analizaron un total de 6 muestras de suero en 5 réplicas, una vez al día durante 5 días por 3 operadores.

A continuación se muestran los datos de un lote representativo:

Muestra	n	Medio conc. (AU/mL)	Intraprueba (repetibilidad)	
			DE	CV
1	75	7,74	0,40	5,2 %
2	75	12,46	0,51	4,1 %
3	75	21,06	0,99	4,7 %
4	75	32,07	1,46	4,6 %
5	75	55,15	1,19	2,2 %
6	75	75,45	2,33	3,1 %

Reproducibilidad: Se analizaron un total de 6 muestras de suero en 5 réplicas, una vez al día durante 5 días por 3 operadores.

A continuación se muestran los resultados de los datos combinados de dos lotes:

Muestra	n	Medio conc. (AU/mL)	Dentro del laboratorio (reproducibilidad)	
			DE	CV %
1	150	7,65	0,46	6,0 %
2	150	12,33	0,74	6,0 %
3	150	21,03	1,60	7,6 %
4	150	31,89	1,98	6,2 %
5	150	54,28	2,70	5,0 %
6	150	75,46	2,63	3,5 %

15.3.5. Linealidad

La linealidad se evaluó en base a CLSI EP-06, “Evaluation of the Linearity of Quantitative Measurement Procedures”. Para la concentración de anticardiolipina IgM mediante el ensayo Anti Cardiolipin Screen, el procedimiento de medición muestra linealidad para el intervalo de 0,82 a 86,88 AU/mL dentro de la desviación de linealidad permitida (ADL) de ±15 %.

15.3.6. Especificidad analítica

Las siguientes sustancias no interfieren con un sesgo de > ±15 % en el ensayo Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgM cuando las concentraciones están por debajo del umbral indicado presentado en la siguiente tabla.

Reactivos que pueden interferir	Límite máximo de concentración
Bilirrubina, conjugada	15 mg/dL
Bilirrubina, no conjugada	15 mg/dL
Hemoglobina	200 mg/dL
Proteína total	10 g/dL
Triglicéridos	500 mg/dL

15.3.7. Estudio en suero-plasma

El estudio de comparación de la matriz de Anti Cardiolipin Screen para la evaluación de anticuerpos de clase IgM se realizó para evaluar la diferencia entre los tipos de tubos (tubos separadores de suero [SST], plasma de heparina de litio, plasma de heparina sódica y plasma K2 EDTA) frente a las muestras de control (tapón rojo para suero, sin aditivos) siguiendo las directrices de CLSI EP9-A3. Se evaluó un total de 20 muestras (16 nativas, 4 con aditivos) para cubrir el intervalo. Se realizó un análisis de regresión lineal sobre los datos comparativos:

Tipo de muestra	Pendiente [IC del 95 %]	Intersección (ng/mL) [IC del 95 %]	Coeficiente de correlación (r)
SST	1,02 [0,94 a 1,09]	0,19 [-1,34 a 1,72]	0,99
Heparina de litio	0,93 [0,82 a 1,04]	0,73 [–1,49 a 2,95]	0,97
Heparina sódica	0,93 [0,84 a 1,01]	0,64 [–1,00 a 2,28]	0,98
EDTA	0,96 [0,86 a 1,05]	0,65 [–1,19 a 2,49]	0,98

16. LÍMITES DE USO

- Como en cualquier procedimiento diagnóstico, los resultados se deberán interpretar junto con los hallazgos clínicos del paciente y otra información de la que el médico disponga.
- Las características de rendimiento de este análisis no se han establecido para una población pediátrica.
- Los anticuerpos heterofílicos en el suero humano pueden presentar reacciones con las inmunoglobulinas reactivas, que interfieren con los inmunoensayos *in vitro*[22]. Los pacientes que se exponen habitualmente a animales o a productos de suero animal pueden ser propensos a esta interferencia y puede que se observen valores anómalos.
- La presencia de inmunocomplejos u otros agregados de inmunoglobulinas en la muestra del

paciente puede causar un mayor nivel de unión no específica y dar como resultado falsos positivos en este ensayo.

17. GESTIÓN DE RESIDUOS

Los reactivos deben eliminarse de acuerdo con la normativa local.

18. BIBLIOGRAFÍA

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19. IDENTIFICADOR DE REVISIÓN

Las adiciones o cambios en las instrucciones de uso se han resaltado en gris.

20. RECLAMACIONES SOBRE PRODUCTOS Y ASISTENCIA TÉCNICA

Para un paciente/usuario/tercero en la Unión Europea y en países con un régimen regulatorio similar: Reglamento (UE) 2017/746 sobre los productos sanitarios para diagnóstico in vitro; si, durante el uso de este dispositivo o como resultado de su uso, se ha producido un incidente grave, informe del mismo al fabricante y/o a su representante autorizado y al organismo regulador nacional.

Puede contactar con el fabricante a través del servicio de atención al cliente o del equipo de asistencia técnica. Los datos de contacto se encuentran a continuación y en el sitio web de la empresa: www.diametra.com.

Ed. 03/2022 **DCM144-2**

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DiaMetra	Packaging Information Sheet	Mod. PIS000-1

IVD	DE ES FR GB IT PT	In vitro Diagnostikum Producto sanitario para diagnóstico In vitro Dispositif medical de diagnostic in vitro In vitro Diagnostic Medical Device Dispositivo medico-diagnostico in vitro Dispositivos medicos de diagnostico in vitro		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
	DE ES FR GB IT PT	Achtung, Begleitdokumente Precaución, consulte los documentos adjuntos Attention, veuillez consulter les documents d'accompagnement Caution, consult accompanying documents Attenzione, consultare la documentazione allegata Atenção,consultar os documentos de acompanhamento	 yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
 yyyy-mm-dd	DE ES FR GB IT PT	Verwendbar bis Establa hasta (usar antes de último día del mes) Utiliser avant (dernier jour du mois indiqué) Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Utilizar (antes ultimo dia do mês)		DE ES FR GB IT PT	Biogefährdung Riesco biológico Risque biologique Biological risk Rischio biologico Risco biológico
	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
 Σ = xx	DE ES FR GB IT PT	Ausreichend für "n" Tests Contenido suficiente para "n" tests Contenu suffisant pour "n" tests Contains sufficient for "n" tests Contenuto sufficiente per "n" saggi Contém o suficiente para "n" testes	CONT	DE ES FR GB IT PT	Inhalt Contenido del estuche Contenu du coffret Contents of kit Contenuto del kit Conteúdo do kit
Max  Min	DE ES FR GB IT PT	Temperaturbereich Límitaciôn de temperatura Limites de température de conservation Temperature limitation Limiti di temperatura Temperaturas limites de conservação	REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Référéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo
	DE ES FR GB IT PT	Vor direkter sonneneinstrahlung schützen Mantener alejado de la luz solar Tenir à l'écart de la lumière du soleil Keep away from sunlight Tenere lontano dalla luce solare Mantenha longe da luz solar			

SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo

- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala

- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado

- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado

- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai

- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage

- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé

- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé

- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs

CE

DiaMetra
care for quality

DCM114-7
Ed. 01/2015

Anti Phospholipid Screen

per analisi di routine

Determinazione quantitativa degli autoanticorpi anti fosfolipidi in siero o plasma umano

IVD

LOT

Vedere etichetta esterna

2°C 8°C

Σ = 96 test

REF DKO114

DESTINAZIONE D'USO

Il kit Anti Phospholipid Screen è un test immunoenzimatico indiretto in fase solida per la misurazione quantitativa degli auto-anticorpi di classe IgG e IgM diretti contro fosfolipidi anionici del siero mediati dalla β2-glicoproteina (Cardiolipina, Fosfatidil serina, Fosfatidil inositolo, Acido fosfatidico, Fosfatidil colina, Lisofosfatidil colina e Fosfatidil etanol-ammina) su siero o plasma umano. Il test si intende per uso diagnostico in vitro come supporto nella diagnosi di aumentato rischio di trombosi in pazienti con Lupus Eritematosus Sistemico (LES) o disordini simili.
Il kit Anti Phospholipid Screen è destinato al solo uso di laboratorio.

1. SIGNIFICATO CLINICO

Il primo studio sugli anticorpi anti-fosfolipidi iniziò nel 1906 quando Wasserman introdusse un test sierologico per la sifilide. Nel 1942 fu scoperto che la componente attiva era un fosfolipide indicato con il nome di Cardiolipina. Negli anni '50 emerse che un elevato numero di persone risultava essere positivo al test per la sifilide senza però presentare alcuna evidenza della malattia. All' inizio si etichettò il fenomeno come una serie di falsi positivi al test per la sifilide, in seguito emerse che in questo gruppo di pazienti vi era una elevata prevalenza di disordini autoimmuni tra cui il Lupus Eritematosus Sistemico (LES) e la Sindrome di Sjögrens.
Il termine Lupus anticoagulante (LA), utilizzato per la prima volta nel 1972, deriva da osservazioni sperimentali nelle quali si osservò un aumento del rischio di trombosi paradossalmente alla presenza di alcuni fattori anticoagulanti; il termine LA non è del tutto corretto poiché la patologia si presenta più frequentemente in pazienti senza lupus ed è associato a trombosi piuttosto che a sanguinamento anormale.
Negli anni più recenti è stato investigato il ruolo di un cofattore , la proteina β2-glicoproteina I (apolipoproteina H) detto anche β2GPI, e le sue interazioni con i fosfolipidi anionici contenuti nel siero/plasma umano. Questo cofattore è una β-globulina con peso molecolare 50 kDa che si trova nel plasma alla concentrazione di circa 200 µg/mL. Si è scoperto che β2GPI è coinvolto nella regolazione della coagulazione del sangue, inibendo la via intrinseca. β2GPI in vivo è associato con sostanze cariche negativamente, quali ad esempio fosfolipidi anionici, eparina e lipoproteine. La regione che lega i fosfolipidi è situata nel suo quinto dominio.
Con l'acronimo "aPL" (anticorpi anti-fosfolipidi) si intendono impropriamente anticorpi diretti contro fosfolipidi carichi negativamente quali la Cardiolipina (CL), Fosfatidil serina (PS) Fosfatidil inositolo (PI) e Acido fosfatidico (PA); secondo una accezione più corretta del termine, vanno intesi come anticorpi anti-fosfolipidi quegli anticorpi diretti contro il complesso tra β2GPI e i fosfolipidi anionici in grado di legarsi al dominio quinto della β2GPI.Tra questi, la Cardiolipina è il fosfolipide usato più comunemente come antigene per dosare gli aPL con metodo ELISA. Anticorpi diretti contro il complesso tra β2GPI e fosfolipidi carichi negativamente, quali Fosfatidil serina (PS) Fosfatidil inositolo (PI) e Acido fosfatidico (PA) vengono misurati nel laboratorio diagnostico.
Alcuni ricercatori hanno suggerito che l'utilizzo della PS al posto della Cardiolipina nei dosaggi ELISA permetterebbe una diagnosi più precisa. Tuttavia, questi anticorpi anti fosfolipidi sono usati meno comunemente e il loro utilizzo aggiuntivo può aumentare la sensibilità clinica nei campioni di pazienti con sospetta Sindrome Anti-fosfolipidica (APS), ma non possono sostituire la determinazione degli autoanticorpi anti Cardiolipina.

2. PRINCIPIO DEL METODO

Il dosaggio Anti Phospholipid Screen si basa sul legame degli anticorpi presenti nel siero e diretti contro i complessi antigenici formati da fosfolipidi anionici (Cardiolipina, Fosfatidil serina, Fosfatidil inositolo, Acido fosfatidico, Fosfatidil colina, Lisofosfatidil colina e Fosfatidil etanol-ammina) e la β2-Glicoproteina; questi complessi sono adsorbiti sulla micropiastra. Gli anticorpi di tipo IgG e IgM diretti verso questi antigeni e presenti nei calibratori, nei controlli, e nei campioni di siero o plasma prediluiti dei pazienti, si legano quindi ai rispettivi antigeni.
Dopo 60 minuti di incubazione la micropiastra viene lavata con Wash Solution per la rimozione delle componenti del siero che non hanno reagito.
Una soluzione di immunoglobuline anti-human IgG (Conjugate IgG, reattivo 4) o anti-human IgM (Conjugate IgM, reattivo 5) riconosce gli anticorpi di classe IgG o IgM (rispettivamente) legati agli antigeni immobilizzati.
Dopo 30 minuti di incubazione, l'eccesso di coniugato enzimatico che non si è legato specificamente, viene rimosso tramite lavaggio con apposito tampone (Wash Solution).
Si aggiunge poi una soluzione substrato cromogenica (TMB Substrate) contenente Tetrametilbenzidina. Dopo 15 minuti di incubazione si blocca lo sviluppo del colore mediante aggiunta della Stop Solution. Il colore della soluzione diventa giallo, e l'intensità di colore sviluppata è direttamente proporzionale alla concentrazione di anticorpi IgG o IgM presenti nel campione originale.
La concentrazione di anticorpi IgG o IgM presenti nel campione è calcolata sulla base di una curva di calibrazione.

3. REATTIVI, MATERIALI E STRUMENTAZIONE

3.1. Reattivi e materiali forniti nel kit

1. Calibrators (5 flaconi, 1,2 mL ciascuno)
Tampone fosfato 0,1M, NaN_3 < 0,1%, siero umano

CAL0	**REF DCE002/11406-0**
CAL1	**REF DCE002/11407-0**
CAL2	**REF DCE002/11408-0**
CAL3	**REF DCE002/11409-0**
CAL4	**REF DCE002/11410-0**

2. Controlli (2 flaconi, 1,2 mL ciascuno, pronti all'uso)
Tampone fosfato 0,1M, NaN_3 < 0,1%, siero umano

Positive Control	**REF DCE045/11402-0**
Negative Control	**REF DCE045/11401-0**

3. Sample Diluent (1 flacone, 100 mL)
Tampone fosfato 0,1M, NaN_3 < 0,1%
REF DCE053-0

4. Conjugate IgG (1 flacone, 15 mL)
Anti h-IgG coniugato con perossidasi di rafano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-G**

5. Conjugate IgM (1 flacone, 15 mL)
Anti h-IgM coniugate con perossidasi di rafano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-M**

6. Coated Microplate (1 micropiastra breakable)
Complessi antigenici di fosfolipidi e β2-Glicoproteina adsorbiti su micropiastra **REF DCE002/11403-0**

7. TMB Substrate (1 flacone, 15 mL)
H_2O_2-TMB (0,26 g/L) *(evitare il contatto con la pelle)*
REF DCE004-0

8. Stop Solution (1 flacone, 15 mL)
Acido solforico 0,15M *(evitare il contatto con la pelle)*
REF DCE005-0

9. 10X Conc. Wash Solution (1 flacone, 50 mL)
Tampone fosfato 0,2M, pH 7.4 **REF DCE054-0**

3.2. Reattivi necessari non forniti nel kit

Acqua distillata.

3.3. Materiale e strumentazione ausiliare

Dispensatori automatici.
Lettore per micropiastre (450 nm, 620-630 nm).

Note

Conservare tutti i reattivi a 2÷8°C, al riparo dalla luce.
Aprire la busta del Reattivo 6 (Coated Microplate) solo dopo averla riportata a temperatura ambiente e chiuderla subito dopo il prelievo delle strip da utilizzare; una volta aperta è stabile fino alla data di scadenza del kit.

4. AVVERTENZE

- Questo test kit è per uso in vitro, da eseguire da parte di personale esperto. Non per uso interno o esterno su esseri Umani o Animali.
- Usare i previsti dispositivi di protezione individuale mentre si lavora con i reagenti forniti.
- Seguire le Buone Pratiche di Laboratorio (GLP) per la manipolazione di prodotti derivati da sangue.
- Materiali di origine animale usati per la preparazione di questo kit sono stati ottenuti da animali sani e le proteine bovine sono state ottenute da paesi non affetti da BSE, ma comunque questi materiali dovrebbero essere usati come potenzialmente contagiosi.
- Tutti i reattivi di origine umana usati nella preparazione dei reagenti sono stati testati e sono risultati negativi per la presenza di anticorpi anti-HIV 1&2, per HbsAg e per anticorpi anti-HCV. Tuttavia nessun test offre la certezza completa dell'assenza di HIV, HBV, HCV o di altri agenti infettivi. Pertanto, i Calibratori ed i Controlli devono essere maneggiati come materiali potenzialmente infettivi.
- Alcuni reagenti contengono piccole quantità di Sodio Azide (NaN_3) o di Proclin 300^R come conservante. Evitare il contatto con la pelle e le mucose.
- La Sodio Azide può essere tossica se ingerita o assorbita attraverso la cute o gli occhi; inoltre, può reagire con le tubature di piombo o rame formando azidi metalliche potenzialmente esplosive. Se si usa un lavandino per eliminare i reagenti, lasciar scorrere grandi quantità di acqua per prevenire la formazione di azidi.
- Il TMB Substrato contiene un irritante, che può essere dannoso se inalato, ingerito o assorbito attraverso la cute. Per prevenire lesioni, evitare l'inalazione, l'ingestione o il contatto con la cute e con gli occhi.
- La Stop Solution è costituita da una soluzione di acido solforico diluito. L'acido solforico è velenoso e corrosivo e può essere tossico se ingerito. Per prevenire possibili ustioni chimiche, evitare il contatto con la cute e con gli occhi.
- Evitare l'esposizione del reagente TMB/H_2O_2 a luce solare diretta, metalli o ossidanti. Non congelare la soluzione.

5. PREACUZIONI

- Si prega di attenersi rigorosamente alla sequenza dei passaggi indicata in questo protocollo. I risultati presentati qui sono stati ottenuti usando specifici reagenti elencati in queste Istruzioni per l'Uso.
- Tutti i reattivi devono essere conservati a temperatura controllata di 2-8°C nei loro contenitori originali. Eventuali eccezioni sono chiaramente indicate. I reagenti sono stabili fino alla data di scadenza se conservati e trattati seguendo le istruzioni fornite.
- Prima dell'uso lasciare tutti i componenti dei kit e i campioni a temperatura ambiente (22-28°C) e mescolare accuratamente.
- Non scambiare componenti dei kit di lotti diversi. Devono essere osservate le date di scadenza riportate sulle etichette della scatola e di tutte le fiale. Non utilizzare componenti oltre la data di scadenza.
- **ATTENZIONE**: **il reagente Coniugato è stato studiato per garantire la massima sensibilità di dosaggio, e pertanto, se non opportunamente usato, può essere contaminato da agenti esterni**; si raccomanda pertanto di utilizzare consumabili (puntali, flaconi, vaschette ecc.) usa e getta. Per dosaggi frazionati, prelevare l'esatta quantità di coniugato necessaria ed evitare di re-introdurre l'eventuale scarto nel flacone originale. Inoltre, **per dosaggi effettuati con l'ausilio di strumentazione automatica e semi-automatica**, si consiglia, prima di utilizzare il coniugato, di effettuare uno step di pulizia della fluidica, assicurandosi che le procedure di lavaggio, deproteinizzazione e decontaminazione siano efficaci nell'evitare la contaminazione del coniugato; questa procedura è fortemente raccomandata quando il kit è processato con analizzatori non dotati di puntali monouso.
  A tale scopo Diametra rende disponibile separatamente un reattivo decontaminante per il lavaggio degli aghi.
- Qualora si utilizzi strumentazione automatica, è responsabilità dell'utilizzatore assicurarsi che il kit sia stato opportunamente validato.
- Un lavaggio incompleto o non accurato dei pozzetti può causare una scarsa precisione e/o un'elevato background. Per migliorare le prestazioni del kit su strumentazione automatica, si consiglia di aumentare il numero di lavaggi.

- Per la riproducibilità dei risultati, è importante che il tempo di reazione di ogni pozzetto sia lo stesso. Per evitare il time shifting durante la dispensazione degli reagenti, il tempo di dispensazione dei pozzetti non dovrebbe estendersi oltre i 10 minuti. Se si protrae oltre, si raccomanda di seguire lo stesso ordine di dispensazione. Se si utilizza più di una piastra, si raccomanda di ripetere la curva di calibrazione in ogni piastra.
- L'aggiunta del TMB Substrato dà inizio ad una reazione cinetica, la quale termina con l'aggiunta della Stop Solution. L'aggiunta del TMB Substrato e della Stop Solution deve avvenire nella stessa sequenza per evitare tempi di reazione differenti.
- Osservare le linee guida per l'esecuzione del controllo di qualità nei laboratori clinici testando controlli e/o pool di sieri.
- Osservare la massima precisione nella ricostituzione e dispensazione dei reagenti.
- Non usare campioni microbiologicamente contaminati, altamente lipemici o emolizzati.
- I lettori di micropiastre leggono l'assorbanza verticalmente. Non toccare il fondo dei pozzetti.

6. PROCEDIMENTO

6.1. Preparazione dei Calibratori (C_0…C_4)

I Calibratori sono pronti all'uso e sono misti, pertanto contengono sia gli anticorpi IgG che IgM. I Calibratori hanno le seguenti concentrazioni:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

Una volta aperti sono stabili 6 mesi a 2-8°C.

6.2. Preparazione del campione

Per l'esecuzione del test si possono utilizzare campioni di siero o di plasma umano. I campioni da utilizzare devono essere limpidi. Si consiglia di evitare contaminazioni dovute a iperlipemia, anche se queste non interferiscono con l'analisi. I campioni possono essere conservati refrigerati a 2-8 °C fino a 5 giorni, oppure conservati a -20°C fino a 6 mesi. Si consiglia di evitare congelamenti e scongelamenti ripetuti dei campioni di siero che potrebbero determinare una perdita variabile dell'attività degli autoanticorpi. Non è raccomandata l'analisi di campioni inattivati al calore.

Tutti i campioni di siero e plasma devono essere prediluiti 1:100 con sample diluent; per esempio 10 µL di siero vengono diluiti con 990 µL di sample diluent.

I Controlli sono pronti all'uso.

6.3. Preparazione della Wash Solution

Prima dell'uso, diluire il contenuto di ogni flacone di soluzione di lavaggio tamponata concentrata (10X) con acqua distillata fino al volume di 500 mL. Per preparare volumi minori rispettare il rapporto di diluizione di 1:10. La soluzione di lavaggio diluita è stabile a 2-8°C per almeno 30 giorni.

Nella wash solution concentrata è possibile osservare la presenza di cristalli, in tal caso agitare a temperatura ambiente fino a completa dissoluzione dei cristalli, per una maggiore precisione diluire tutto il flacone della soluzione di lavaggio concentrata a 500 mL avendo cura di trasferire anche i cristalli, poi agitare fino a completa dissoluzione.

6.4. Procedimento

- **Portare tutti i reagenti a temperatura ambiente (22-28°C) per almeno 30 minuti.** Al termine del dosaggio riporre immediatamente tutti i reagenti a 2-8°C: evitare lunghi periodi a temperatura ambiente.
- Le strisce di pozzetti non utilizzate devono essere rimesse immediatamente nella busta richiudibile contenente il materiale essicante e conservate a 2-8°C.
- Per evitare potenziali contaminazioni microbiche e/o chimiche non rimettere i reagenti inutilizzati nei flaconi originali.
- Al fine di aumentare l'accuratezza dei risultati del test è necessario operare in doppio, allestendo due pozzetti per ogni punto della curva di calibrazione (C_0-C_4), due per ogni Controllo, due per ogni Campione ed uno per il Bianco.

Reagenti	**Calibratore**	**Campione /Controlli**	**Bianco**
Calibratore C_0-C_4	**100 µL**		
Controlli		**100 µL**	
Campione diluito		**100 µL**	
Incubare 60 minuti a temperatura ambiente (22-28°C). Allontanare la miscela di reazione, lavare i pozzetti 3 volte con 300 µL di wash solution diluita. **Nota importante**: ad ogni step di lavaggio, agitare delicatamente la piastra per 5 secondi e successivamente rimuovere l'eccesso di soluzione di lavaggio sbattendo delicatamente la micropiastra capovolta su fogli di carta assorbente. **Lavaggi automatici**: se si utilizza strumentazione automatica effettuare almeno 5 lavaggi.			
Conjugate (IgG or IgM)	**100 µL**	**100 µL**	
Incubare 30 minuti a temperatura ambiente (22-28°C). Allontanare la miscela di reazione, lavare i pozzetti 3 volte con 300 µL di wash solution diluita. **Lavaggi**: seguire le stesse indicazioni del punto precedente.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubare 15 minuti a temperatura ambiente al riparo dalla luce (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Agitare delicatamente la micropiastra. Leggere l'assorbanza (E) a 450 nm contro una lunghezza d'onda di riferimento di 620-630 nm oppure contro il Bianco entro 5 minuti.			

7. CONTROLLO DI QUALITA'

- Il Controllo Positivo per anti-fosfolipidi deve essere incluso ogni volta che si esegue il test per assicurare che tutti i reagenti ed il test funzionino in modo corretto.
- Poichè il Controllo è prediluito, esso non rappresenta un controllo procedurale per le tecniche di diluizione utilizzate per i campioni.
- Ulteriori sieri di controllo possono essere preparati raccogliendo un pool di sieri umani, aliquotandolo e conservandolo a < -20°C.
- Perchè i risultati del test siano considerati validi, tutti i seguenti criteri devono essere soddisfatti. Se anche uno solo non rientra nei valori specificati, i risultati non dovrebbero essere considerati validi ed il test dovrebbe essere ripetuto:

- Il Controllo Positivo serve per controllare un'eventuale malfunzionamento dei reagenti e non assicura la precisione in corrispondenza del valore soglia del test.
- Il test è valido solo se la densità ottica a 450 nm del Controllo Positivo come pure quelle dei calibratori (C_0-C_4) coincidono con gli intervalli corrispondenti indicati nel Certificato di Controllo di Qualità incluso nel kit.

8. CALCOLO DEI RISULTATI

Per il kit Anti Phospholipid Screen il metodo di scelta per il trattamento dei risultati è una elaborazione 4 parametri con assi lin-log per densità ottica e concentrazione rispettivamente. Inoltre si possono utilizzare un'approssimazione spline e coordinate log-log. Tuttavia si raccomanda di utilizzare una curva lin-log.
Innanzitutto occorre calcolare la media delle densità ottiche relative ai calibratori. Utilizzare un foglio di carta lin-log e tracciare le densità ottiche medie di ogni calibratore verso la rispettiva concentrazione. Disegnare la curva che approssima nel modo migliore tutti i punti di calibrazione. I punti dei calibratori possono anche essere collegati con segmenti di linea retta. La concentrazione dei campioni incogniti può essere determinata per interpolazione dalla curva di calibrazione.

9. VALORI DI RIFERIMENTO

In uno studio sui valori normali eseguito con campioni di siero provenienti da donatori sani sono stati determinati i seguenti intervalli di normalità con il test Anti Phospholipid Screen:

	IgG (GPL AU/mL)	IgM (MPL AU/mL)
Normale	< 10	< 10
Elevato	≥ 10	≥ 10

È importante tenere presente che la determinazione di un range di valori attesi in un dato metodo per una popolazione "normale" è dipendente da molteplici fattori, quali la specificità e sensibilità del metodo in uso, e la popolazione in esame. Perciò ogni laboratorio dovrebbe considerare i range indicati dal Fabbricante come un'indicazione generale e produrre range di valori attesi propri basati sulla popolazione indigena dove il laboratorio risiede.
I risultati positivi dovrebbero essere verificati relativamente allo stato clinico del paziente. Inoltre, ogni decisione relativa alla terapia dovrebbe essere presa individualmente. Si raccomanda che ogni laboratorio stabilisca i sui propri intervalli normale e patologico di anticorpi anti fosfolipidi serici.

10. LIMITAZIONI DEL TEST

La presenza nel campione di complessi immuni o di altri aggregati di immunoglobuline può determinare delle reazioni aspecifiche con conseguenti risultati falsi positivi.

11. PARAMETRI CARATTERISTICI

11.1. Precisione e riproducibilità

La precisione e la riproducibilità sono state valutate testando due campioni in due esperimenti diversi con due lotti di kit differenti.
Le operazioni di dispensazione e lavaggio sono state eseguite da un operatore manualmente.
I risultati in termini di deviazione standard e coefficiente di variazione sono riportati di seguito:

	IgG			
Campione	1		2	
	SD	CV%	SD	CV%
Intra-assay	1.03	5.9	1.31	7.4
Inter-assay	0.26	9.2	5.25	11.7
	IgM			
Campione	1		2	
	SD	CV%	SD	CV%
Intra-assay	0.61	7.6	1.97	5.9
Inter-assay	0.15	7.1	2.98	6.6

11.2. Sensibilità:

La sensibilità clinica del saggio Anti Phospholipid Screen IgG è 92,3%.
La sensibilità clinica del saggio Anti Phospholipid Screen IgM è 68,8%.

11.3. Specificità:

La specificità clinica del saggio Anti Phospholipid Screen IgG è 84,6%.
La specificità clinica del saggio Anti Phospholipid Screen IgM è > 99,9%.

11.4. Limite di rilevabilità:

La minor concentrazione che può essere distinta dal Calibratore zero è di circa 0,3 AU/mL per IgG e 0,16 AU/mL per IgM.

12. DISPOSIZIONI PER LO SMALTIMENTO

I reagenti devono essere smaltiti in accordo con le leggi locali.

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3. Domke N., Siegert G.: Phospholipidantikörper und ihre klinische Bedeutung. Zeitschrift für Klinische Medizin 16: 1399-1401, 1988.
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CE

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DCM114-7
Ed. 01/2015

Anti Phospholipid Screen

for routine analysis

Quantitative determination of auto-antibodies against phospholipids in human serum or plasma

IVD

LOT
See external label

2°C 8°C

Σ = 96 test

REF DKO114

INTENDED USE

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

1. CLINICAL SIGNIFICANCE

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

2. PRINCIPLE

Anti Phospholipid Screen test is based on the binding of antibodies in human serum directed against the antigenic complex between anionic phospholipids (Cardiolipin, Phosphatidyl serine, Phosphatidyl inositol, Phosphatidic acid, Phosphatidyl choline, Lyso-phosphatidyl choline, Phosphatidyl ethanolamine) and β2-Glycoprotein; these complexes are coated on the microplate. Any antibody of IgG class or IgM class in calibrators, controls or prediluted patient samples binds to its respective antigen.
After 60 minutes incubation, the microplate is washed with wash buffer for removing non-reactive serum components. An anti-human IgG conjugate solution (Conjugate IgG, reactive 4) or an anti-human IgM conjugate solution (Conjugate IgM, reactive 5) recognize IgG class or IgM class antibodies, respectively, bound to the immobilized antigens.
After a 30 minutes incubation any excess enzyme conjugate which is not specifically bound is washed away with the wash buffer.
A chromogenic substrate solution containing TMB is then dispensed into the wells. After a 15 minutes incubation the color development is stopped by adding the stop solution. The solutions color change into yellow. The amount of color is directly proportional to the concentration of IgG or IgM antibodies present in the original sample.
The concentration of IgG or IgM antibodies in the original sample is calculated through a calibration curve.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrators (5 vials, 1,2 mL each)
Phosphate buffer 0,1M, NaN_3 < 0,1%, human serum

CAL0	**REF DCE002/11406-0**
CAL1	**REF DCE002/11407-0**
CAL2	**REF DCE002/11408-0**
CAL3	**REF DCE002/11409-0**
CAL4	**REF DCE002/11410-0**

2. Controls (2 vials, 1,2 mL each, ready to use)
Phosphate buffer 0,1M, NaN_3 < 0,1%, human serum

Positive Control	**REF DCE045/11402-0**
Negative Control	**REF DCE045/11401-0**

3. Sample Diluent (1 vial, 100 mL)
Tampone fosfato 0,1 M NaN_3 < 0,1%
REF DCE053-0

4. Conjugate IgG (1 vial, 15 mL)
Anti h-IgG conjugate with horseradish peroxidise (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-G**

5. Conjugate IgM (1 vial, 15 mL)
Anti h-IgM conjugate with horseradish peroxidise (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-M**

6. Coated Microplate (1 breakable microplate)
Antigenic phospholipid and β2-Glicoprotein complexes coated on the microplate **REF DCE002/11403-0**

7. TMB Substrate (1 vial, 15 mL)
H_2O_2 -TMB (0,26 g/L) *(avoid any skin contact)*
REF DCE004-0

8. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0,15M *(avoid any skin contact)*
REF DCE005-0

9. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0,2M pH 7.4 **REF DCE054-0**

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

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

Notes

Store all reagents between 2÷8°C in the dark.
Open the bag of reagent 6 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit.

4. WARNINGS

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

5. PRECAUTIONS

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

Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of Calibrators (C_0…C_4)

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

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

Once opened, the Calibrators are stable 6 months at 2-8°C.

6.2. Sample Preparation

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

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

The Controls are ready to use.

6.3. Wash Solution Preparation

Dilute the contents of each vial of the buffered wash solution concentrate (10X) with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.

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

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay store immediately the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results, prepare two wells for each point of the calibration curve (C_0-C_4), two for each Control, two for each sample, one for Blank.

Reagents	Calibrator	Sample/ Controls	Blank
Calibrator C_0-C_4	**100 µL**		
Controls		**100 µL**	
Diluted Sample		**100 µL**	
Incubate 60 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Important note**: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel. **Automatic washer**: if you use automated equipment, wash the wells at least 5 times.			
Conjugate (IgG or IgM)	**100 µL**	**100 µL**	
Incubate 30 minutes at room temperature (22-28°C). Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution. **Washing**: follow the same indications of the previous point.			
TMB Substrate	**100 µL**	**100 µL**	**100 µL**
Incubate 15 minutes in the dark at room temperature (22-28°C).			
Stop Solution	**100 µL**	**100 µL**	**100 µL**
Shake the microplate gently. Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.			

7. QUALITY CONTROL

- The anti-phopholipids Positive Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
- Because Positive Control is prediluted, it do not control for procedural methods associated with dilution of specimens.
- Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at < -20°C.
- In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test should be considered invalid and the assay repeated:

- The Positive Control are intended to monitor for substantial reagent failure and they will not ensure precision at the assay cut-off.
- This test is only valid if the optical density at 450 nm for Positive Control as well as for the Calibrator (C_0-C_4) complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit.

8. RESULTS

For Anti Phospholipid Screen kit a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed-Spline Approximation and log-log coordinates are also suitable. However we recommend using a Lin-Log Plot.

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may

also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

9. REFERENCE VALUES

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

	IgG (GPL AU/mL)	IgM (MPL AU/mL)
Normal	< 10	< 10
Elevated	≥ 10	≥ 10

Please pay attention to the fact that the determination of a range of expected values for a “normal” population in a given method is dependent on many factors, such as specificity and sensitivity of the method used and type of population under investigation. Therefore each laboratory should consider the range given by the Manufacurer as a general indication and produce their own range of expected values based on the indigenous population where the laboratory works.
Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of seric Ab-Anti-Phospholipid.

10. LIMITATIONS OF PROCEDURE

The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay

11. PERFORMANCE AND CHARACTERISTICS

11.1. Precision and reproducibility

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

	IgG			
Sample	1		2	
	SD	CV%	SD	CV%
Intra-assay	1.03	5.9	1.31	7.4
Inter-assay	0.26	9.2	5.25	11.7
	IgM			
Sample	1		2	
	SD	CV%	SD	CV%
Intra-assay	0.61	7.6	1.97	5.9
Inter-assay	0.15	7.1	2.98	6.6

11.2. Sensitivity

The clinicl sensitivity of Anti Phoshpolipids Screen IgG assay is 92,3%.
The clinicl sensitivity of Anti Phoshpolipids Screen IgM assay is 68,8%.

11.3. Specificity

The clinical specificity of Anti Phoshpolipids Screen IgG assay is 84,6%.
The clinical specificity of Anti Phoshpolipids Screen IgM assay is > 99,9%.

11.4. Detection Limit

The lowest concentration that can be distinguished from Calibrator zero is 0.3 AU/mL for IgG and 0.16 AU/mL for IgM.

12. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations..

BIBLIOGRAPHY

1. Hughes G.R.V., Harris E.N., Gharavi A.E.: The Anticardiolipin Syndrome. J. Rheumat. 13, 3: 486-489, 1986.
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DCM114-7
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Anti Phospholipid Screen

para análisis de rutina

Determinación cuantitativa de autoanticuerpos anti-fosfolípidos en suero o plasma

IVD | **LOT** Ver etiqueta externa | 2°C 8°C | Σ = 96 ensayos | **REF DKO114**

USO PREVISTO

El kit de análisis anti-fosfolípidos es un ensayo inmunoenzimático en fase sólida para la determinación cuantitativa en suero o plasma humano de autoanticuerpos de tipo IgG e IgM contra los fosfolípidos aniónicos mediados por la β2 glicoproteína, incluyendo cardiolipina, fosfatidil serina, fosfatidil inositolo, ácido fosfatídico, fosfatidil colina, lisofosfatidil colina y fosfatidil etanolamina. El ensayo está diseñado para uso diagnóstico *in vitro*, como coadyuvante en el diagnóstico de mayor riesgo de trombosis en pacientes con lupus eritematoso sistémico (LES) o trastornos similares.
El kit Anti Phospholipid Screen está destinado al uso en laboratorio exclusivamente.

1. SIGNIFICADO CLÍNICO

El primer estudio sobre anticuerpos anti-fosfolípidos data de 1906, cuando Wasserman introdujo un análisis serológico para la sífilis. En 1942 se descubrió que el componente activo era un fosfolípido indicado con el nombre de cardiolipina. En los años 50 se observó que un gran número de personas era positiva al análisis de la sífilis aunque no presentaba ninguno de los síntomas de la enfermedad. Al principio, el fenómeno fue catalogado como una serie de resultados falsamente positivos al análisis de sífilis; más tarde se advirtió que en este grupo de pacientes había una gran preponderancia de trastornos autoinmunes, entre ellos el lupus eritematoso sistémico (LES) y el síndrome de Sjögrens.
El término *lupus anticoagulante* (LA), utilizado por primera vez en 1972, deriva de observaciones experimentales en las que se advirtió un aumento del riesgo de trombosis, paradójicamente en presencia de algunos factores anticoagulantes; el término LA no es del todo incorrecto, porque la patología es más frecuente en pacientes sin lupus y va asociada a trombosis antes que a sangrado anormal.
En años más recientes, se investigó el papel de un cofactor, la proteína β2-glicoproteina I (apolipoproteína H) también llamada β2GPI, y su interacción con los fosfolípidos aniónicos contenidos en el suero o plasma humanos. Este cofactor es una β-globulina con peso molecular 50 kDa que se encuentra en el plasma en concentración aproximada de 200 μg/mL. Se descubrió que β2GPI interviene en la regulación de la coagulación de la sangre, inhibiendo la vía intrínseca. *In vivo,* la β2GPI está asociada a sustancias de carga negativa, por ejemplo fosfolípidos aniónicos, heparina y lipoproteínas. La región que liga los fosfolípidos está ubicada en su quinto dominio.
Con el acrónimo "aPL" (anticuerpos anti-fosfolípidos) se identifican impropiamente anticuerpos contra fosfolípidos de carga negativa como la cardiolipina (CL), la fosfatidil serina (PS), el fosfatil inositolo (PI) y el ácido fosfatídico (PA); según una acepción más correcta del término, se entiende por anticuerpos anti-fosfolípidos aquellos anticuerpos dirigidos contra el complejo formado por la β2GPI y los fosfolípidos aniónicos capaces de ligarse al dominio quinto de la β2GPI. De éstos, la cardiolipina es el fosfolípido utilizado más frecuentemente como antígeno para dosificar los aPL con el método ELISA. En el laboratorio clínico se miden los anticuerpos contra el complejo de β2GPI y fosfolípidos de carga negativa, tales como fosfatidil serina (PS), fosfatidil inositol (PI) y ácido fosfatídico (PA).
Algunos investigadores sugieren que el empleo de la PS en lugar de la cardiolipina en los ensayos ELISA permitiría un diagnóstico más exacto. Sin embargo, estos anticuerpos anti-fosfolípidos se utilizan con menos frecuencia aunque su empleo puede aumentar la sensibilidad clínica en las muestras de pacientes en que se sospecha la síndrome anti-fosfolipídica, pero no pueden reemplazar la determinación de los autoanticuerpos anti-cardiolipina.

2. PRINCIPIO DEL MÉTODO

El ensayo de detección de anti-fosfolípidos se basa en el ligado de los anticuerpos presentes en el suero contra los complejos antigénicos formados por fosfolípidos aniónicos (cardiolipina, fosfatidil serina, fosfatidil inositolo, ácido fosfatídico, fosfatidil colina, lisofosfatidil colina y fosfatidil etanolamina) y la β2 glicoproteína; estos complejos están inmovilizados en la microplaca. Los anticuerpos de tipos IgG y IgM dirigidos contra estos antígenos, presentes en los calibradores, controles y muestras de suero o plasma diluidas de pacientes, se ligan a sus respectivos antígenos. Tras 60 minutos de incubación, se lava la microplaca con solución de lavado para eliminar los componentes del suero inactivos. Una solución de inmunoglobulinas anti-humanas IgG (IgG conjugado, reactivo 4) o anti-humano IgM (IgM Conjugado, reactivo 5) reconoce la IgG o IgM (respectivamente) unido a los antígenos inmovilizados.
Tras 30 minutos de incubación, el exceso de conjugado enzimático no ligado específicamente se elimina mediante lavado con solución de lavado. A continuación se añade una solución sustrato cromogénica que contiene tetrametilbenzidina (sustrato TMB). Se incuba 15 minutos y se para el desarrollo del color añadiendo la solución de parada. El color de la solución se vuelve amarillo y la intensidad de color desarrollada es directamente proporcional a la concentración de anticuerpos IgG o IgM de la muestra original.

3. REACTIVOS, MATERIALES E INSTRUMENTACIÓN

3.1. Reactivos y materiales suministrados en el kit

1. Calibradores (5 frascos, 1,2 mL cada uno)
Tampón fosfato 0,1 M, NaN_3 < 0,1%, suero humano

CAL0	**REF DCE002/11406-0**
CAL1	**REF DCE002/11407-0**
CAL2	**REF DCE002/11408-0**
CAL3	**REF DCE002/11409-0**
CAL4	**REF DCE002/11410-0**

2. Controles (2 frascos, 1,2 mL cada uno)
Tampón fosfato 0,1 M, NaN_3 < 0,1%, suero humano

Control negativo	**REF DCE045/11401-0**
Control positivo	**REF DCE045/11402-0**

3. Diluyente de muestra (1 frasco, 100 mL)
Tampón fosfato 0,1 M, NaN_3 < 0,1%
REF DCE053-0

4. Conjugado IgG (1 frasco, 15 mL)
Anti h-IgG conjugado con peroxidasa de rabano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-G**

5. Conjugado IgM (1 frasco, 15 mL)
Anti h-IgM conjugado con peroxidasa de rabano (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/11402-M**

6. Microplaca recubierta
(1 microplaca rompible con el complejo antigénico de fosfolípidos y β2 glicoproteína adsorbidos)
REF DCE002/11403-0

7. Substrato TMB (1 frasco, 15 mL)
H_2O_2-TMB (0,26 g/L) *(evitar el contacto con la piel)*
REF DCE004-0

8. Solución de parada (1 frasco, 15 mL)
0,15M ácido sulfúrico *(evitar el contacto con la piel)*
REF DCE005-0

9. Solución de lavado conc. 10X (1 frasco, 50 mL)
Tampón fosfato 0,2M pH 7.4 **REF DCE054-0**

3.2. Reactivos necesarios no suministrados en el kit

Agua destilada.

3.3. Material e instrumentación auxiliares

Dispensadores automáticos.
Lector de microplacas (450 nm, 620-630 nm).

Notas

Conservar todos los reactivos a 2÷8°C, protegidos de la luz. Abrir la bolsa del reactivo 6 (microplaca recubierta) solo cuando se encuentre a temperatura ambiente y cerrarla inmediatamente después de extraer las tiras que se vayan a utilizar; una vez abierta, permanece estable hasta la fecha de caducidad del kit.

4. ADVERTENCIAS

- Este kit de ensayo está previsto para usarse in vitro y por personal experto. No es para uso interno o externo en humanos o animales.
- Usar los equipos de protección individual previstos al trabajar con los reactivos suministrados.
- Siga las Buenas Prácticas de Laboratorio (GLP) en el manejo de las muestras sanguíneas y sus derivados.
- Todos los reactivos de origen humano usados en la preparación de los Calibradores y de los controles se han comprobado y han resultado negativos para la presencia de anticuerpos anti-VIH 1 y 2, para HbsAg y para anticuerpos anti-VHC. Sin embargo, ningún ensayo ofrece seguridad absoluta de la ausencia de VIH, VHB, VHC o de otros agentes infecciosos. Por lo tanto, los Calibradores y lo control positivo deben manipularse como material potencialmente infeccioso.
- Materiales de origen animal utilizadas para la elaboración de este kit se obtuvieron a partir de animales sanos y de las proteínas de bovino se obtuvieron de los países no afectados por la EEB, pero estos materiales se debe utilizar como potencialmente infecciosos.
- Algunos reactivos contienen pequeñas cantidades de Azida de Sodio (NaN_3) o Proclin 300^R como conservante. Evite el contacto con la piel y las mucosas.
- La Azida de Sodio, usada como conservante, puede ser tóxica si se ingiere o se absorbe a través de la piel o de los ojos; además, puede reaccionar con las tuberías de plomo o cobre formando azidas metálicas potencialmente explosivas. Dejar que corra gran cantidad de agua, si se usa un lavabo para eliminar los reactivos, para prevenir la formación de azidas.
- El cromógeno TMB contiene un irritante que puede ser dañino si se inhala, se ingiere o se absorbe a través de la piel. Para prevenir lesiones, evitar la inhalación, la ingestión o el contacto con la piel y con los ojos.
- La solución de parada está formada por una solución de ácido sulfúrico diluido. El ácido sulfúrico es venenoso y corrosivo, y puede ser tóxico si se ingiere. Para prevenir posibles quemaduras químicas, evitar el contacto con la piel y con los ojos.
- Evite la exposición de los reactivos TMB/H_2O_2 a la luz solar directa, metales u oxidantes. No congelar la solución.

5. PRECAUCIONES

- Respetar rigurosamente la secuencia de los pasos indicados en este protocolo. Los resultados aquí presentados se han obtenido utilizando los reactivos específicos que figuran en estas instrucciones de uso.
- Todos los reactivos deben conservarse a una temperatura controlada de 2-8°C en sus recipientes originales. Todas las excepciones están claramente marcados. Los reactivos son estables hasta la fecha de caducidad cuando se almacenan y manipulan de acuerdo con las instrucciones proporcionadas.
- Antes del uso, esperar hasta que todos los componentes del kit y las muestras se encuentren a temperatura ambiente (22-28°C) y mezclar cuidadosamente.
- No mezclar componentes de kits de lotes distintos. Se debe observar la fecha de caducidad indicada en la etiqueta de la caja y de todas las ampollas. No usar componentes después de la fecha de caducidad.
- **ATENCIÓN**: **se ha estudiado el reactivo conjugado para garantizar la máxima sensibilidad en la determinación y, por lo tanto, si no se usa adecuadamente, podría contaminarse por agentes externos**; se recomienda utilizar consumibles (puntas, frascos, bandejas, etc.) desechables. Para determinaciones fraccionadas, tomar la cantidad necesaria exacta de conjugando y evitar volver a introducir los posibles restos en el frasco original. Además, **para determinaciones realizadas con la ayuda de instrumentación automática y semiautomática**, se recomienda, antes de usar el conjugado, realizar una fase de limpieza de la fluídica, asegurándose de que los procedimientos de lavado, desproteinización y descontaminación resulten eficaces para evitar la contaminación del conjugado; este procedimiento se recomienda especialmente cuando el kit se procesa con analizadores que no están dotados de puntas monouso. Para tal fin, Diametra pone a su disposición por separado un reactivo descontaminante para el lavado de las agujas.

- Si utiliza un equipo automático, es responsabilidad del usuario asegurar que el equipo ha sido debidamente validada.
- Un lavado incompleto o impreciso y la aspiración insuficiente del líquido de los pocillos ELISA pueden causar una precisión pobre y/o un elevado fondo. Para mejorar el rendimiento del kit en los sistemas automatizados, se recomienda aumentar el número de lavados.
- Para la reproducibilidad de los resultados, es importante que el tiempo de reacción sea igual para cada pocillo. El tiempo de dispensación de los pocillos no debe superar los 10 minutos; si se prolongara más allá de los 10 minutos, respétese el orden de dispensación. si utiliza más de una placa, se recomienda repetir la curva de calibración en cada plato.
- Al añadir el substrato TMB inicia una reacción cinética que termina al agregar la solución de parada. Tanto el sustrato como la solución de parada deben agregarse en la misma secuencia para evitar diferentes tiempos de reacción.
- Observar las directrices para la ejecución del control de calidad en los laboratorios clínicos al comprobar controles y/o pool de sueros.
- Observar la máxima precisión en la reconstitución y dispensación de los reactivos.
- No use muestras con contaminación microbiana, altamente lipémicas o hemolizadas.
- Los lectores de microplacas leen las DO verticalmente, por tanto no debe tocarse el fondo de los pocillos.

6. PROCEDIMIENTO

6.1. Preparación de los Calibradores (C_0…C_4)

Los Calibradores están listos para usar y son mixtos, es decir, contienen tanto los anticuerpos IgG como IgM. Los Calibradores están listos para usarse y tienen las siguientes concentraciones:

	C_0	C_1	C_2	C_3	C_4
AU/mL	0	5	10	20	80

Una vez abiertos, los Calibratores permanecen estables 6 meses conservados a 2-8°C.

6.2. Preparación de la muestra

Para realizar el ensayo se pueden usar muestras de suero o plasma humano. Las muestras que se van a usar deben estar limpias. Se recomienda evitar la contaminación por hiperlipemia, aunque esta no interfiera con el análisis. Las muestras pueden conservarse refrigeradas a 2-8°C durante 5 días, o a -20°C hasta 6 meses. Se recomienda no congelar y descongelar repetidamente las muestras de suero o plasma, puesto que esto podría provocar una pérdida variable de la actividad de los autoanticuerpos. No se recomienda el análisis de muestras inactivadas por calor.

Todas las muestras de suero o plasma deben prediluirse 1:100 con diluyente de muestras. por ejemplo 10 µL de suero o plasma pueden diluirse con 990 µL de diluyente de muestras.

Los controles están listos para usar.

6.3. Preparación de la solución de lavado

Antes del uso, diluir el contenido de cada frasco de solución de lavado tamponada concentrada (10x) con agua destilada hasta un volumen de 500 mL. Para preparar volúmenes menores, respetar la relación de dilución de 1:10. La solución de lavado diluida se mantiene estable a 2-8°C durante al menos 30 días. En la solución de lavado concentrada es posible observar la presencia de cristales. En ese caso, agitar a temperatura ambiente hasta que los cristales se disuelvan por completo. Para una mayor precisión, diluir todo el frasco de la solución de lavado concentrada a 500 mL, teniendo cuidado para transferir también los cristales y, a continuación, agitar hasta que se disuelvan por completo.

6.4. Procedimiento

- **Esperar hasta que todos los reactivos se encuentren a temperatura ambiente (22-28°C) durante al menos 30 minutos.** Al final del ensayo inmediatamente poner todos los reactivos a 2-8°C para evitar largos periodos a temperatura ambiente.
- Las tiras de pocillos no utilizados se deben guardar de inmediato en la bolsa desechable que contiene desecantes y almacenarse a 2-8°C.
- Para evitar la contaminación microbiana y/o química no regrese porciones de reactivos no usados en los viales originales.
- Para aumentar la precisión de los resultados de la prueba es necesario trabajar en duplicado: preparar dos pocillos para cada punto de la curva de calibración (C_0-C_4), dos para cada control, dos para cada muestra, uno para el blanco.

Reactivos	Calibrador	Muestra/ Controles	Blanco
Calibrador C_0-C_4	**100 µL**		
Controles		**100 µL**	
Muestra diluida		**100 µL**	
Incubar 60 minutos a temperatura ambiente (22-28°C). Retirar la mezcla de reacción y lavar los pocillos tres veces con 300 µL de solución de lavado diluida. **Nota importante**: agite suavemente la placa durante 5 segundos en cada paso del lavado. Después del último lavado asegúrese haber eliminado completamente la solución de lavado de los pozos, invierta la placa y golpéela repetidas veces contra una servilleta de papel absorbente. **Lavados automático**: si está utilizando una lavadora automática, lavar los pocillos al menos 5 veces.			
Conjugado (IgG o IgM)	**100 µL**	**100 µL**	
Incubar 30 minutos a temperatura ambiente (22-28 °C). Retirar la mezcla de reacción y lavar los pocillos tres veces con 300 µL de solución de lavado diluida. **Lavados**: siga las mismas instrucciones del punto anterior.			
Substrato TMB	**100 µL**	**100 µL**	**100 µL**
Incubar 15 minutos a temperatura ambiente (22-28°C), en la oscuridad.			
Solución de parada	**100 µL**	**100 µL**	**100 µL**
Agitar la microplaca con cuidado. Leer la absorbancia (E) a 450 nm frente una segunda lectura de referencia a 620-630 nm o frente al blanco entre 5 minutos.			

7. CONTROL DE CALIDAD

- Los controles positivo y negativo deben incluirse cada vez que se realice el ensayo para asegurar que todos los reactivos y el ensayo funcionen de forma correcta.
- Puesto que los controles están prediluidos, no representan un control de procedimiento para las técnicas de dilución usadas para las muestras.
- Se pueden preparar sueros de control adicionales recogiendo un pool de sueros humanos, dividiéndolo en alícuotas y conservándolo a < -20 °C.
- Para que los resultados del ensayo se consideren válidos, se deben cumplir todos los criterios siguientes. Aunque solo uno no se encuentre dentro de los valores especificados, los resultados no deberán considerarse válidos y el ensayo deberá repetirse:
  - La absorbancia del control positivo debe ser mayor que la del control negativo.
  - El control negativo y el positivo sirven para controlar un eventual malfuncionamiento de los reactivos y no aseguran la precisión en correspondencia con el valor límite del ensayo.
  - El ensayo es válido solo si la densidad óptica a 450 nm del control negativo y del control positivo, así como las de los calibradores (C_0-C_4), coinciden con los intervalos correspondientes indicados en el Certificado de control de calidad incluido en el kit.

8. CÁLCULO DE LOS RESULTADOS

Para Anti Phospholipid Screen, el método de elección para el tratamiento de los resultados es un procesamiento de 4 parámetros con ejes lin-log para la densidad óptica y la concentración respectivamente. Además, se pueden usar una aproximación spline y coordenadas log-log. Sin embargo, se recomienda usar una curva Lin-Log.
En primer lugar, calcular la media de las densidades ópticas relativas a los calibradores. Usar una hoja de papel lin-log y trazar las densidades ópticas medias de cada calibrador frente a la respectiva concentración. Dibujar la curva que mejor se aproxime a todos los puntos de calibración. Los puntos de los calibradores también pueden unirse con segmentos de línea recta. La concentración de las muestras desconocidas puede determinarse por interpolación de la curva de calibración.

9. VALORES DE REFERENCIA

En un estudio sobre los valores normales realizado con muestras de suero procedentes de donantes sanos se han determinado los siguientes intervalos de normalidad con el ensayo Anti-Phospholipid Screen:

	IgG (GPL AU/mL)	**IgM (MPL AU/mL)**
Normal	< 10	< 10
Alto	≥ 10	≥ 10

Es importante señalar que la determinación de un rango de valores esperados en un método dado para una población "normal" depende de muchos factores, tales como la especificidad y sensibilidad del método en uso, y la población en estudio. Por lo tanto, cada laboratorio debe considerar el intervalo especificado por el fabricante como una guía general y producir su propio rango de valores calculados en base al estadístico obtenido por el laboratorio, donde reside la población local.
Los resultados positivos deben verificarse con relación al estado clínico del paciente. Además, cada decisión relativa a la terapia debe tomarse individualmente. Se recomienda que cada laboratorio establezca sus propios intervalos normal y patológico de anticuerpos anti-fosfolipidos sérica.

10. LIMITACIONES DEL ENSAYO

La presencia en la muestra de complejos inmunes o de otros agregados de inmunoglobulinas puede dar lugar a reacciones no específicas con resultados falsos positivos.

11. PARÁMETROS CARACTERÍSTICOS

11.1. Precisión y reproducibilidad

La precisión y la reproducibilidad se evaluaron analizando ocho duplicados de dos muestras positivas en dos ensayos diferentes con dos lotes de kits diferentes.
La dispensación y el lavado las efectuó manualmente un operador.
Los resultados de desviación Calibración y coeficiente de variación se indican a continuación:

	IgG			
Muestra	1		2	
	SD	CV%	SD	CV%
Intra-ensayo	1.03	5.9	1.31	7.4
Entre-ensayos	0.26	9.2	5.25	11.7
	IgM			
Muestra	1		2	
	SD	CV%	SD	CV%
Intra-ensayo	0.61	7.6	1.97	5.9
Entre-ensayos	0.15	7.1	2.98	6.6

11.2. Sensibilidad

La sensibilidad clínica del ensayo anti-fosfolípidos IgG es de 92,3%.
La sensibilidad clínica del ensayo anti-fosfolípidos IgM es un 68,8%.

11.3. Especificidad

La especificidad clínica del ensayo anti-fosfolípidos IgG es de 84,6%.
La especificidad clínica del ensayo anti-fosfolípidos IgM es un 100%.

11.4. Límite de detección:

La concentración mínima que puede distinguirse del Calibración cero es de aproximadamente 0,3 AU/mL para IgG y 0.16 AU/mL para IgM.

12. DISPOSICIONES PARA LA ELIMINACIÓN

Los reactivos deben eliminarse de acuerdo con las leyes locales.

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DiaMetra	Packaging Information Sheet	Mod. PIS000-1

IVD	DE ES FR GB IT PT	In vitro Diagnostikum Producto sanitario para diagnóstico In vitro Dispositif medical de diagnostic in vitro In vitro Diagnostic Medical Device Dispositivo medico-diagnostico in vitro Dispositivos medicos de diagnostico in vitro		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
	DE ES FR GB IT PT	Achtung, Begleitdokumente Precaución, consulte los documentos adjuntos Attention, veuillez consulter les documents d'accompagnement Caution, consult accompanying documents Attenzione, consultare la documentazione allegata Atenção,consultar os documentos de acompanhamento	 yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
 yyyy-mm-dd	DE ES FR GB IT PT	Verwendbar bis Establa hasta (usar antes de último día del mes) Utiliser avant (dernier jour du mois indiqué) Use by (last day of the month) Utilizzare prima del (ultimo giorno del mese) Utilizar (antes ultimo dia do mês)		DE ES FR GB IT PT	Biogefährdung Riesco biológico Risque biologique Biological risk Rischio biologico Risco biológico
	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
 Σ = xx	DE ES FR GB IT PT	Ausreichend für "n" Tests Contenido suficiente para "n" tests Contenu suffisant pour "n" tests Contains sufficient for "n" tests Contenuto sufficiente per "n" saggi Contém o suficiente para "n" testes	CONT	DE ES FR GB IT PT	Inhalt Contenido del estuche Contenu du coffret Contents of kit Contenuto del kit Conteúdo do kit
Max  Min	DE ES FR GB IT PT	Temperaturbereich Límitaciôn de temperatura Limites de température de conservation Temperature limitation Limiti di temperatura Temperaturas limites de conservação	REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Référéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo
	DE ES FR GB IT PT	Vor direkter sonneneinstrahlung schützen Mantener alejado de la luz solar Tenir à l'écart de la lumière du soleil Keep away from sunlight Tenere lontano dalla luce solare Mantenha longe da luz solar			

SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI

Nessuna reazione colorimetrica del saggio

- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)

- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala

- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato

- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato

- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS

No colorimetric reaction

- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)

- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers

- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run

- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)

- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo

- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)

- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala

- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado

- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado

- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai

- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)

- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage

- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé

- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé

- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs

Anti-LKM-1 ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EA 1321-9601 G	LKM-1	IgG	Ag-coated microplate wells	96 x 01 (96)

Indications: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human antibodies of the IgG class against liver-kidney microsomes (LKM) in serum or plasma for the diagnosis of inexplainable increase in transaminases, suspected autoimmune hepatitis.

Application: According to the simplified diagnostic criteria by EM Hennes and colleagues *(International Autoimmune Hepatitis Group*) published in 2008, the detection of autoantibodies against LKM belongs to the routine investigations performed to diagnose autoimmune hepatitis. Antibodies against LKM-1 are mostly observed in children, but may be also present in adult patients with AIH. For delimitation from a virus hepatitis, the parallel determination of the other autoantibodies associated with AIH, such as ANA, pANCA, ASMA or antibodies against LC-1 and SLA/LP is recommended.

Principles of the test: The test kit contains microtiter strips each with 8 break-off reagent wells coated with LKM-1. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgG antibodies (also IgA and IgM) will bind to the antigens. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) catalysing a colour reaction.

Contents of the test kit:

Component	Colour	Format	Symbol
1. Microplate wells coated with antigens 12 microplate strips each containing 8 individual break-off wells in a frame, ready for use	---	12 x 8	STRIPS
2. Calibrator 1 200 RU/ml (IgG, human), ready for use	dark red	1 x 2.0 ml	CAL 1
3. Calibrator 2 20 RU/ml (IgG, human), ready for use	red	1 x 2.0 ml	CAL 2
4. Calibrator 3 2 RU/ml (IgG, human), ready for use	light red	1 x 2.0 ml	CAL 3
5. Positive control (IgG, human), ready for use	blue	1 x 2.0 ml	POS CONTROL
6. Negative control (IgG, human), ready for use	green	1 x 2.0 ml	NEG CONTROL
7. Enzyme conjugate peroxidase-labelled anti-human IgG (rabbit), ready for use	green	1 x 12 ml	CONJUGATE
8. Sample buffer ready for use	light blue	1 x 100 ml	SAMPLE BUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASH BUFFER 10x
10. Chromogen/substrate solution TMB/H_2O_2, ready for use	colourless	1 x 12 ml	SUBSTRATE
11. Stop solution 0.5 M sulphuric acid, ready for use	colourless	1 x 12 ml	STOP SOLUTION
12. Test instruction	---	1 booklet	
13. Quality control certificate	---	1 protocol	

LOT Lot description
IVD In vitro diagnostic medical device
CE
Storage temperature
Unopened usable until

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) approx. 30 minutes before use. After first use, the reagents are stable until the indicated expiry date if stored at +2°C to +8°C and protected from contamination, unless stated otherwise below.

- **Coated wells:** Ready for use. Tear open the resealable protective wrapping of the microplate at the recesses above the grip seam. Do not open until the microplate has reached room temperature to prevent the individual strips from moistening. Immediately replace the remaining wells of a partly used microplate in the protective wrapping and tightly seal with the integrated grip seam (Do not remove the desiccant bag).
  Once the protective wrapping has been opened for the first time, the wells coated with antigens can be stored in a dry place and at a temperature between +2°C and +8°C for 4 months.

- **Calibrators and controls:** Ready for use. The reagents must be mixed thoroughly before use.

- **Enzyme conjugate:** Ready for use. The enzyme conjugate must be mixed thoroughly before use.

- **Sample buffer:** Ready for use.

- **Wash buffer:** The wash buffer is a 10x concentrate. If crystallisation occurs in the concentrated buffer, warm it to 37°C and mix well before diluting. The quantity required should be removed from the bottle using a clean pipette and diluted with deionised or distilled water (1 part reagent plus 9 parts distilled water).
  For example: For 1 microplate strip, 5 ml concentrate plus 45 ml water.
  The working strength wash buffer is stable for 4 weeks when stored at +2°C to +8°C and handled properly.

- **Chromogen/substrate solution:** Ready for use. Close the bottle immediately after use, as the contents are sensitive to light. The chromogen/substrate solution must be clear on use. Do not use the solution if it is blue coloured.

- **Stop solution:** Ready for use.

Storage and stability: The test kit has to be stored at a temperature between +2°C to +8°C. Do not freeze. Unopened, all test kit components are stable until the indicated expiry date.

Waste disposal: Patient samples, calibrators, controls and incubated microplate strips should be handled as infectious waste. All reagents must be disposed of in accordance with local disposal regulations.

Warning: The calibrators and controls of human origin have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the agent sodium azide in a non-declarable concentration. Avoid skin contact.

Preparation and stability of the patient samples

Samples: Human serum or EDTA, heparin or citrate plasma.

Stability: Patient samples to be investigated can generally be stored at +2°C to +8°C for up to 14 days. Diluted samples should be incubated within one working day.

Sample dilution: Patient samples are diluted **1:101** in sample buffer. For example: dilute 10 µl of sample in 1.0 ml sample buffer and mix well by vortexing (sample pipettes are not suitable for mixing).

NOTE: Calibrators and controls are prediluted and ready for use, do not dilute them.

Incubation

For **semiquantative analysis** incubate **calibrator 2** along with the positive and negative controls and patient samples. For **quantitative analysis** incubate **calibrators 1, 2 and 3** along with the positive and negative controls and patient samples.

(Partly) manual test performance

Sample incubation: (1st step)
Transfer 100 µl of the calibrators, positive and negative controls or diluted patient samples into the individual microplate wells according to the pipetting protocol. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Manual: Empty the wells and subsequently wash 3 times using 300 µl of working strength wash buffer for each wash.
Automatic: Wash reagent wells 3 times with 450 µl of working strength wash buffer (program setting: e.g. TECAN Columbus Washer "Overflow Modus").

Leave the wash buffer in each well for 30 to 60 seconds per washing cycle, then empty the wells. After washing (manual and automated tests), thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with the openings facing downwards to remove all residual wash buffer.

Note: Residual liquid (> 10 µl) remaining in the reagent wells after washing can interfere with the substrate and lead to false low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short residence times) can lead to false high extinction values.
Free positions on the microplate strip should be filled with blank wells of the same plate format as that of the parameter to be investigated.

Conjugate incubation: (2nd step)
Pipette 100 µl of enzyme conjugate (peroxidase-labelled anti-human IgG) into each of the microplate wells. Incubate for **30 minutes** at room temperature (+18°C to +25°C).

Washing:
Empty the wells. Wash as described above.

Substrate incubation: (3rd step)
Pipette 100 µl of chromogen/substrate solution into each of the microplate wells. Incubate for **15 minutes** at room temperature (+18°C to +25°C), protect from direct sunlight.

Stopping the reaction:
Pipette 100 µl of stop solution into each of the microplate wells in the same order and at the same speed as the chromogen/substrate solution was introduced.

Measurement:
Photometric measurement of the colour intensity should be made at a **wavelength of 450 nm** and a reference wavelength between 620 nm and 650 nm **within 30 minutes of adding the stop solution**. Prior to measuring, slightly shake the microplate to ensure a homogeneous distribution of the solution.

Test performance using fully automated analysis devices

Sample dilution and test performance are carried out fully automatically using an analysis device. The incubation conditions programmed in the respective software authorised by EUROIMMUN may deviate slightly from the specifications given in the ELISA test instruction. However, these conditions were validated in respect of the combination of the EUROIMMUN Analyzer I, Analyzer I-2P or the DSX from Dynex and this EUROIMMUN ELISA. Validation documents are available on enquiry.
Automated test performance using other fully automated, open system analysis devices is possible. However, the combination should be validated by the user.

Pipetting protocol

	1	2	3	4	5	6	7	8	9	10	11	12
A	**C 2**	**P 6**	**P 14**	**P 22**			**C 1**	**P 4**	**P 12**	**P 20**		
B	**pos.**	**P 7**	**P 15**	**P 23**			**C 2**	**P 5**	**P 13**	**P 21**		
C	**neg.**	**P 8**	**P 16**	**P 24**			**C 3**	**P 6**	**P 14**	**P 22**		
D	**P 1**	**P 9**	**P 17**				**pos.**	**P 7**	**P 15**	**P 23**		
E	**P 2**	**P 10**	**P 18**				**neg.**	**P 8**	**P 16**	**P 24**		
F	**P 3**	**P 11**	**P 19**				**P 1**	**P 9**	**P 17**			
G	**P 4**	**P 12**	**P 20**				**P 2**	**P 10**	**P 18**			
H	**P 5**	**P 13**	**P 21**				**P 3**	**P 11**	**P 19**			

The pipetting protocol for microtiter strips 1-4 is an example for the **semiquantitative analysis** of 24 patient samples (P 1 to P 24).
The pipetting protocol for microtiter strips 7-10 is an example for the **quantitative analysis** of 24 patient samples (P 1 to P 24).

The calibrators (C 1 to C 3), the positive (pos.) and negative (neg.) controls, and the patient samples have each been incubated in one well. The reliability of the ELISA test can be improved by duplicate determinations for each sample.
The wells can be broken off individually from the strips. This makes it possible to adjust the number of test substrates used to the number of samples to be examined and minimises reagent wastage.
Both positive and negative controls serve as internal controls for the reliability of the test procedure. They should be assayed with each test run.

Calculation of results

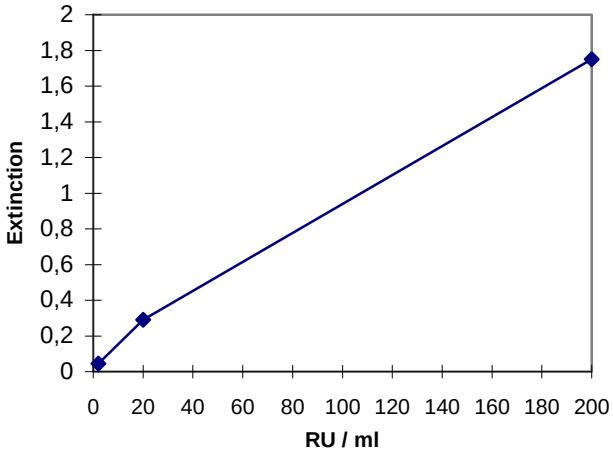
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator 2. Calculate the ratio according to the following formula:

$$\frac{\text{Extinction of the control or patient sample}}{\text{Extinction of calibrator 2}} = \text{Ratio}$$

EUROIMMUN recommends interpreting results as follows:

Ratio <1.0:	**negative**
Ratio ≥1.0:	**positive**

Quantitative: The standard curve from which the concentration of antibodies in the serum samples can be taken is obtained by point-to-point plotting of the extinction values measured for the 3 calibration sera against the corresponding units (linear/linear). Use “point-to-point” plotting for calculation of the standard curve by computer. The following plot is an example of a typical calibration curve. Please do not use this curve for the determination of antibody concentrations in patient samples.



If the extinction for a patient sample lies above the value of calibrator 1 (200 RU/ml), the result should be reported as ">200 RU/ml". It is recommended that the sample be re-tested at a dilution of e.g. 1:400. The result in RU/ml read from the calibration curve for this sample must then be multiplied by a factor of 4.

The upper limit of the normal range **(cut-off)** recommended by EUROIMMUN is 20 relative units (RU)/ml. EUROIMMUN recommends interpreting results as follows:

<20 RU/ml:	**negative**
≥20 RU/ml:	**positive**

For duplicate determinations the mean of the two values should be taken. If the two values deviate substantially from one another, EUROIMMUN recommends to retest the samples.

For diagnosis, the clinical picture of the patient always needs to be taken into account along with the serological findings.

Test characteristics

Calibration: As no international reference serum exists for antibodies against LKM-1, the calibration is performed in relative units (RU).

For every group of tests performed, the extinction values of the calibrators and the relative units and/or ratio determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.

The binding activity of the antibodies and the activity of the enzyme used are temperature-dependent. It is therefore recommended using a thermostat in all three incubation steps. The higher the room temperature during the incubation steps, the greater will be the extinction values. Corresponding variations apply also to the incubation times. However, the calibrators are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.

Antigen: The reagent wells are coated with recombinant cytochrome P450 IID6 which constitutes the specific target antigen for antibodies against LKM1.

Linearity: The linearity of the Anti-LKM-1 ELISA (IgG) was determined by assaying 4 serial dilutions of different patient samples. The coefficient of determination R^2 for all sera was > 0.95. The Anti-LKM-1 ELISA (IgG) is linear at least in the tested concentration range (2 RU/ml to 194 RU/ml).

Detection limit: The lower detection limit is defined as the mean value of an analyte-free sample plus three times the standard deviation and is the smallest detectable antibody titer. The lower detection limit of the Anti-LKM-1 ELISA (IgG) is 1.4 RU/ml.

Cross reactivity: This ELISA showed no cross reactivity.

Interference: Haemolytic, lipaemic and icteric samples showed no influences on the result up to a concentration of 10 mg/ml for haemoglobin, 20 mg/ml for triglycerides and 0.4 mg/ml for bilirubin in this ELISA.

Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation (CV) using 3 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

Intra-Assay Variation, n = 20		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	55	3.0
2	96	2.7
3	158	2.3

Inter-Assay Variation, n = 4 x 6		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	58	3.2
2	96	3.7
3	160	2.5

Specificity and sensitivity: 18 patient samples suffering from autoimmune hepatitis and 489 patient samples from a reference laboratory were investigated with the EUROIMMUN Anti-LKM-1 ELISA (IgG). The EUROIMMUN-IIFT (IgG) was used as a reference method. The ELISA has a specificity of 99.4% and a sensitivity of 100% with reference to the EUROIMMUN IIFT.

Serum panel (n = 507)		**IIFT (rat liver/rat kidney)**	
		positive	negative
Anti-LKM-1 ELISA	positive	27	3
	negative	0	477

A patient sample which reacted positive in ELISA and negative in IIFT belongs to a patient with characterised AIH.

Reference range: The levels of the anti-LKM-1 antibodies (IgG) were analysed with this EUROIMMUN ELISA in a panel of 200 healthy blood donors. With a cut-off of 20 RU/ml, 0.5% of the blood donors were anti-LKM-1 positive.

Clinical significance

In Western Europe the incidence of AIH is 1.9 cases per 100,000 in-habitants per year. Untreated, AIH soon develops into liver cirrhosis. However, if low-dose immunosuppressive therapy is started early enough and continued lifelong, patients have a normal life expectancy.

Circulating autoantibodies have come to play a significant role in the diagnosis of AIH. Antibodies against the following antigens are associated with AIH: soluble liver antigen/liver-pancreas antigen (SLA/LP), cell nuclei (ANA), nDNA, smooth muscles (SMA, the most important target antigen being F actin), liver-kidney microsomes (LKM-1, target antigen: cytochrome P450 IID6), liver cytosolic antigen type 1 (LC-1, target antigen: formiminotransferase cyclo-deaminase) and granulocytes (pANCA). Anti-mito-chondrial antibodies (AMA) are also investigated in this context to exclude the possibility of primary biliary cirrhosis (PBC). AIH is sometimes classified according to the antibody status, i.e., subtype I (ANA, SMA), subtype II (antibodies against LKM-1 and LC-1), or subtype III (antibodies against SLA/LP). However, this classification is probably neither clinically nor therapeutically or prognostically relevant, since 10 to 20% of patients with PBC develop secondary AIH (overlap). In these cases the same autoantibodies as in AIH are frequently detected.

Autoantibodies against LKM-1 (LKM-1, antigen: cytochrome P450 IID6) are present in 1% of adults with AIH. In children they are more common. Antibodies against LKM-1 are also found in 1 to 2% of patients with hepatitis C-positive serology.

The highest diagnostic accuray currently available for AIH is probably provided by the various EUROIMMUN enzyme immunoassays that detect autoantibodies against SLA/LP. Although SLA/LP autoantibodies have a prevalence of only 10 to 30% in AIH patients, the predictive value is nearly 100%. Every positive anti-SLA/LP result essentially indicates AIH (provided the relevant clinical symptoms are also present).

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IVD

REF 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	Sufficient for ... determinations
LOT	Batch code
	Use by
	Temperature limitation
	Keep away from sunlight
	Do not reuse
	Date of manufacture
CE	CE marked according to 98/79/EC
	Consult electronic Instructions For Use
519_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	Enzyme Conjugate
TMB	TMB Substrate
STOP	Stop solution
WASH	Wash Buffer
RTU	Ready to use
50 x	50 x concentrate

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	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.

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

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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_2018-01-02_3* Reason for revision: *Definition of symbols used and symbols updated*

1. **100 µl** Standards, Kontrollen und verdünnte Patientenproben pipettieren
   → **30 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und
   3 mal mit **300 µl** Waschpuffer waschen
2. **100 µl** Enzymkonjugatlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
   → Inhalt der Platte verwerfen und
   3 mal mit **300 µl** Waschpuffer waschen
3. **100 µl** Substratlösung pipettieren
   → **15 Minuten** bei Raumtemperatur inkubieren
4. **100 µl** Stopplösung zugeben
   → Platte **5 Minuten** stehenlassen
   → Bei **450 nm** messen