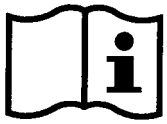
Product information



Userś Manual

Sclero-Pro IgG ELISA

Enzyme Immunoassay for the determination of IgG antibodies against eight different cellular and nuclear antigens in human serum

REF AE29002

Σ 96

RUO

For Research Use Only – Not for Use in Diagnostic Procedures

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1 INTENDED USE

This ELISA is a solid phase enzyme immunoassay for the separate qualitative detection of IgG antibodies against eight different cellular and nuclear antigens in human serum. The wells are separately coated with recombinant human 100 kDa PM-Scl, 70 kDa U1-snRNP, SS-B, SS-A 52 kDa, SS-A 60 kDa, Scl 70, centromere protein B (CenpB), Jo-1 and highly purified native human Sm. For research use only, not for use in diagnostic procedures.

2 INTRODUCTION

Anti-nuclear antibodies (ANA) are an important tool for the differential diagnosis of systemic rheumatic diseases. Indirect immunofluorescence test (IFT) on eucaryotic cells like HeLa has been the established method for the detection of ANAs. Single antibody specificities are distinguished by fluorescence patterns but more specific testing by ELISAs employing the target antigens are available too for a simple and reliable differentiation of ANAs.

Antibodies against:

- PM-Scl (Polymyositis Scleroderma antigen; 100 kDa) are directed against a 100 kDa protein of a nucleolar multiprotein complex consisting of 11 proteins. Anti-PM-Scl antibodies are found in individualss with connective tissue diseases. They define a subset of individuals with myositis in overlap with systemic sclerosis.
- U1-snRNP is directed to the 70 kDa protein of U1 snRNP. They are pathognomic for MCTD but do also occur in SLE. A high titer of antibodies against this antigen is typical for the Sharp-Syndrome.
- Sm (Smith antigen) are directed against core proteins (B,B`, D1-D3, E, F, G) of small nuclear ribonucleoproteins (snRNPs). Anti-Sm as well as antibodies against double stranded DNA (dsDNA) are highly specific for SLE and thus are included in the classification criteria for SLE.
- SS-A (Ro; soluble cytoplasmic and/or nuclear ribonucleoproteins of 52 kDa and 60 kDa) and antibodies against SS-B (La; 48 kDa protein associated with RNA polymerase III) are mainly found in high titers for primary and secondary Sjögren`s syndrome but also in SLE, congenital heartblock and neonatal lupus.
- Scl-70 are directed against DNA-topoisomerase I. They are highly specific for systemic scleroderma and give a hint for a severe course.
- CenpB (80kDa centromere protein B) are typical for the CREST-Syndrome (69% positive), which is a more protracted type of systemic sclerosis
- Jo-1 are directed against histidyl-tRNA synthetase (cytoplasmic protein involved in protein biosynthesis) and are found in 20-40 % of individuals with polymyositis and dermatomyositis

3 PRINCIPLE OF THE TEST

Principle of the test

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

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4 REAGENTS

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	2 x 1.8ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	2 x 1.8ml	Blue	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15ml	Red	Red	Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
MATERIALS REQUIRED, BUT NOT PROVIDED				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

5 STORAGE CONDITIONS

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

6 WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.[25]
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. For research use only, not for use in diagnostic procedures.

7 INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

8 SAMPLE COLLECTION AND PREPARATION

1. The use of SERUM samples is required for this test.
2. Samples should be collected using standard venipuncture techniques. Remove serum from the coagulated or packed cells within 60 minutes after collection.
3. Samples which cannot be assayed within 24 hours of collection should be frozen at –20 °C or lower, and will be stable for up to six months.
4. Avoid grossly hemolytic (bright red), lipemic (milky), or turbid samples (after centrifugation).
5. Samples should not be repeatedly frozen and thawed prior to testing. DO NOT store in “frost free” freezers, which may cause occasional thawing. Samples which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

9 PROCEDURAL NOTES

1. Pipetting Recommendations (single and multi-channel). Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

10 PREPARATION OF REAGENTS AND SAMPLES

All reagents should be brought to room temperature (18 °C - 25 °C) before use.

Dilute concentrated reagents:

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

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

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

Samples:

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

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

Washing:

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

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

Automated washing:

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

Manual washing:

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

Microplates:

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

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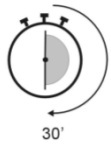
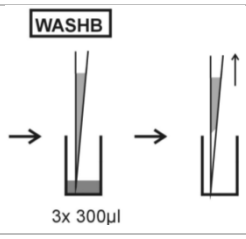
11 ASSAY PROCEDURE

We suggest pipetting calibrators, controls and samples as follows:

Antigen		1	2	3	4...	
U1-70-RNP	A	CC	NC	P1	P2	P3
Sm	B	CC	NC	P1	P2	P3
SS-A	C	CC	NC	P1	P2	P3
SS-B	D	CC	NC	P1	P2	P3
Scl70	E	CC	NC	P1	P2	P3
PmScl	F	CC	NC	P1	P2	P3
CenpB	G	CC	NC	P1	P2	P3
Jo-1	H	CC	NC	P1	P2	P3

NC: negative control
CC: cut-off calibrator

P1: Sample 1
P2: Sample 2
P3: Sample 3

Step	*Description*	
1.	Ensure preparations from step 10 above have been carried out prior to pipetting.	
2.	Use the following steps in accordance with quantitative/ qualitative interpretation results desired:	
	CONTROLS & SAMPLES	
3.		Pipette into the designated wells as described above, 100 µl of either: Cut-off Calibrator (CC) for *QUALITATIVE* interp. and 100 µl of each of the following: • Negative control (NC) and Positive control (PC), and • Sample diluted serum (P1, P2...)
4.		Incubate for 30 minutes at 20-32°C/68-89.6°F.
5.		Wash 3x with 300 µl washing buffer (diluted 1:50).

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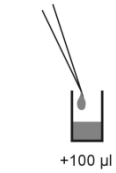
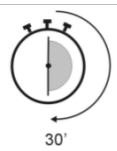
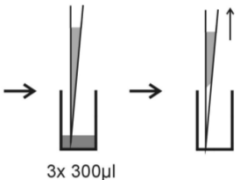
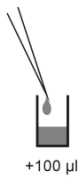
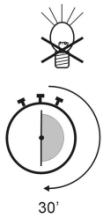
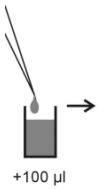
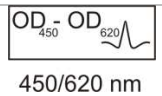
	CONJUGATE	
6.	CONJ 	Pipette 100 µl conjugate into each well.
7.		Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.	WASHB 	Wash 3x with 300 µl washing buffer (diluted 1:50).
	SUBSTRATE	
9.	SUB 	Pipette 100 µl TMB substrate into each well.
10.		Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from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

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12 INTERPRETATION

Read the optical density of the cut-off calibrator and the samples. Multiply the OD of the cut-off calibrator by the parameterspecific factor, provided with the lot specific QC certificate. Compare all sample ODs with the calculated parameter OD cut-off value. For qualitative interpretation we recommend to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative

Sclero Profil	O.D. 450/620 nm
Negative Control	0.033
Cut-off Calibrator	0.550

Example of interpretation

We recommend pipetting cut-off calibrator in parallel for each run.

QC-Certificate:	Jo-1 Factor		**0.95**
Measured:	$OD_{\text{Cut-off Calibrator}}$ (Jo-1)		**0.550**
Calculation:	$OD_{\text{Cut-off Parameter}}$ (Jo-1)	0.550 x 0.95=	**0.5225**

Negative:	OD_{sample}	< 0.8 x $OD_{\text{Cut-off Parameter}}$	= 0.8 x 0.5225	=0.418
Positive:	OD_{sample}	> 1.2 x $OD_{\text{Cut-off Parameter}}$	= 1.2 x 0.5225	=0.627
Equivocal:	0.418 ≤	OD_{Sample}		≤ 0.627

ID Nr.	Sample	OD - Calculation	Interpretation
	OD Jo-1		
1	0.99	> 0.627	--->Positive
2	0.49	≥ 0.418 und ≤ 0.627	--->Equivocal
3	0.27	< 0.418	--->Negative

Do not use this example for interpreting sample results!

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

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

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

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

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

For semi-quantification of the results, each sample OD-value can be expressed by the Index-Value. The Index-Value is calculated by dividing the sample-OD by the cut-off parameter:

$$\textbf{Index Value} = \frac{\textbf{OD (sample)}}{\textbf{OD (cut-off calibrator)}}$$

Negative:		**Index Value**	**< 0.8**
Equivocal:	**0.8≤**	**Index Value**	**≤ 1.2**
Positive:		**Index Value**	**>1.2**

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13 TECHNICAL DATA

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

14 PERFORMANCE DATA

14.1 Specificity and Sensitivity

The microplate is coated with highly purified and/or recombinant antigens (100 kDa PM-Scl, 70 kDa U1-snRNP, SS-B, SS-A 52 kDa, SS-A 60 kDa, Scl 70, centromere protein B (CenpB), Jo-1 and Sm). No crossreactivities to other autoantigens have been found.

Since Sclero Pro consists of various antigens, the values are shown in a table respectively

	Sensitivity
U1-snRNP	100% for mixed connective tissue disease
SS-A	80% for Sjögren´s syndrome
Scl 70	20-48% 20-48% for systemic scleroderma
Jo-1	25% for polymyositis and dermatomyositis
CenpB	up to 80% for CREST-Syndrom
Sm	10-30% for SLE
PM-Scl	5-10% dermatomyositis

14.2 Linearity

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

Sample No.	Dilution Factor	measured concentration (OD-Ratio)	expected concentration (OD-Ratio)	Recovery (%)
1	1 / 100	4.4	4.5	97.8
	1 / 200	2.4	2.3	104.3
	1 / 400	1.2	1.2	100.0
	1 / 800	0.6	0.6	100.0
2	1 / 100	3.7	3.8	97.4
	1 / 200	1.8	1.9	94.7
	1 / 400	0.95	1.0	95.0
	1 / 800	0.55	0.5	110.0

14.3 Precision

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

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Intra-assay		
Sclero-Pro	**Mean OD Ratio**	**CV (%)**
RNP-70	1.5	0.5
Sm	2.1	0.9
SSA 52/60	1.8	1.1
SSB	3.2	0.7
Scl-70	3.5	0.5
PMScl	1.9	0.9
CenpB	2.6	1.5
Jo-1	3.4	1.6

Inter-assay		
Sclero-Pro	**Mean OD Ratio**	**CV (%)**
RNP-70	1.5	0.5
Sm	2.6	0.9
SSA 52/60	3.1	1.5
SSB	2.5	1.5
Scl-70	1.9	1.4
PMScl	3.6	0.6
CenpB	2.2	2.0
Jo-1	1.9	1.7

14.4 Calibration

This Sclero-Pro IgG ELISA is calibrated against reference sera from the CDC (Centers for Disease Control and Prevention) Atlanta.

15 LITERATURE

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Schmolke M, Oppermann M, Helmke K, Guder WG. Antibody determination against ENA- a challenge for the routine laboratory. Poster P59, 5 th Dresden Symposium on Autoantibodies, 2000.

Tan EM, (1989). Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. Adv. Immunol 44: 93-151.

Manufactured for :
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SYMBOLS USED WITH IBL-AMERICA ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

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