Product information

Users Manual

Anti PM-Scl ELISA

Enzyme Immunoassay for the determination of antibodies against PM-Scl in human serum

REF AE29001

Σ 96

RUO

For Research Use Only – Not for Use in Diagnostic Procedures

1   INTENDED USE

This anti PM-Scl ELISA is a solid phase enzyme immunoassay employing recombinant human 100 kDa PM-Scl for the quantitative and qualitative detection of antibodies against PM-Scl in human serum. For research use only, not for use in diagnostic procedures.

2   INTRODUCTION

The polymysositis/scleroderma i. e. PM/Scl antigen was first described by Reichlin and Mathioli in 1976. PM/Scl antibodies can be found in sera from individuals suffering from polymyositis/scleroderma overlap syndrome (PM/Scl).

The two major PM-Scl antigens are proteins of 100kDa and 75kDa, termed PM-Scl 100 and PM-Scl 75, respectively. Most anti-PM/Scl-positive sera are reactive with the 100 kDa protein, while some also recognize the 75 kDa protein.

The PM-Scl 75 and PM-Scl 100 antigens are part of a multiprotein particle also termed PM-Scl (formerly PM-1). The cellular function of the complete PM-Scl particle has long been an enigma.

Recently, the human PM/Scl complex was shown to be related to the yeast exosome, a complex which functions in very diverse pathways, all dealing with RNA degradation, such as mRNA turnover, rRNA processing and sn(o)RNA biogenesis.

3   PRINCIPLE OF THE TEST

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.

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	1 x 1.5ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5ml	Red	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	1 x 1.5ml	Blue	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	6 x 1.5ml	White	Yellow *	Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15ml	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.
* Color increasing with concentration				

MATERIALS REQUIRED, BUT NOT PROVIDED
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

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.

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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).

11 ASSAY PROCEDURE

We suggest pipetting calibrators, controls and samples as follows:

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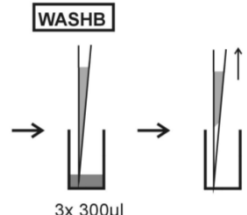
	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	…	
H	Cal D	NC	…	

For *QUALITATIVE* interpretation

	1	2	3	4...
A	NC	P2		
B	NC	P2		
C	CC	P3		
D	CC	P3		
E	PC	…		
F	PC	…		
G	P1	…		
H	P1	…		
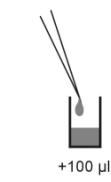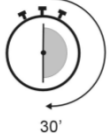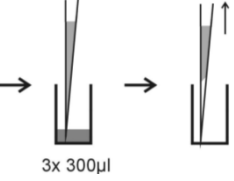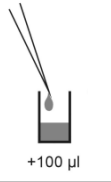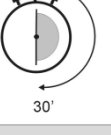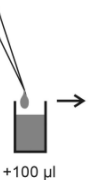
Cal.A: calibrator A   Cal.D: calibrator D   PC: positive control   P1: sample 1
Cal.B: calibrator B   Cal.E: calibrator E   NC: negative control   P2: sample 2
Cal.C: calibrator C   Cal.F: calibrator F   CC: cut-off calibrator   P3: sample 3

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: a. Calibrators (CAL.A to CAL.F) for *QUANTITATIVE* or b. Cut-off Calibrator (CC) for *QUALITATIVE* interp. and 100 µl of each of the following: • Negative control (NC) and Positive control (PC), and • Samples diluted serum (P1, P2...) +100 µl
4.	Incubate for 30 minutes at 20-32°C/68-89.6°F. 30'
5.	WASHB Wash 3x with 300 µl washing buffer (diluted 1:50). 3x 300µl

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	CONJUGATE	
6.	CONJ +100 µl	Pipette 100 µl conjugate into each well.
7.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.	WASHB 3x 300µl	Wash 3x with 300 µl washing buffer (diluted 1:50).

	SUBSTRATE	
9.	SUB +100 µl	Pipette 100 µl TMB substrate into each well.
10.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.

	STOP	
11.	STOP +100 µl	Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.	5'	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.

12 INTERPRETATION

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

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

Example of a standard curve
Do not use this example for interpreting your results!

Calibrators	OD 450/620 nm	CV % (Variation)
0 U/ml	0.070	1.0
3 U/ml	0.176	1.9
10 U/ml	0.322	2.1
30 U/ml	0.636	3.9
100 U/ml	1.271	0.4
300 U/ml	2.138	0.8

Example of calculation

Sample	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.859/0.883	0.871	51.0
P 02	0.499/0.498	0.499	20.4

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.
For lot specific data, see enclosed quality control leaflet. Laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

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

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

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

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

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

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

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 Analytical Sensitivity

Testing sample buffer 30 times with this ELISA gave an analytical sensitivity of 1.0 U/ml.

14.2 Specificity and Sensitivity

The microplate is coated with recombinant human 100 kDa PM-Scl. No cross reactivities to other auto-antigens have been found. This ELISA therefore exhibits a diagnostic specificity of 90% and a diagnostic sensitivity of 80%.

14.3 Linearity

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

Sample No.	Dilution Factor	Measured (U/ml)	Expected (U/ml)	Recovery (%)
1	1 / 100	126.0	123.0	102.4
	1 / 200	62.8	61.5	102.1
	1 / 400	31.9	30.8	103.6
	1 / 800	16.8	15.4	109.1
2	1 / 100	78.5	76.0	103.3
	1 / 200	36.9	38.0	97.1
	1 / 400	17.4	19.0	91.6
	1 / 800	10.0	9.5	105.3

14.4 Precision

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

Intra-assay		
Sample No.	Mean (U/ml)	CV (%)
1	128.0	2.8
2	69.0	3.0
3	21.0	4.3

Inter-assay		
Sample No.	Mean (U/ml)	CV (%)
1	125.0	2.9
2	66.0	3.5
3	19.0	4.1

14.5 Calibration

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

15 LITERATURE

Reichlin M and Mattioli (1976). Description of a serological reaction characteristic of polymyositis.Clin. Immunol. Immunopathol. 5 (1):12-20.

Brouwer R, Vree Egberts WTM, Hengstman GJD, Raijmakers R; van Engelen BGM, Seelig HP, Renz M, Mierau R, Genth W, Pruijn GJM and van Venrooij WJ (2002). Autoantibodies directed to novel components of the PM/Scl complex, the human exosome. Arthritis Research 4 (2): 134-138.

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
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
Σ	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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