Anti-PM-Scl ELISA (IgG)
Test instruction

ORDER NO.	ANTIBODIES AGAINST	IG CLASS	SUBSTRATE	FORMAT
EA 1584-9601 G	PM-Scl (PM-1)	IgG	Ag-coated microplate wells	96 x 01 (96)

Indication: Overlap syndrome: symptoms of polymyositis (PM), dermatomyositis (DM), and progressive systemic sclerosis (Scl).

Principle of the test: The ELISA test kit provides a semiquantitative or quantitative in vitro assay for human autoantibodies of the IgG class against PM-Scl (PM-1) in serum or plasma. The test kit contains microtiter strips each with 8 break-off reagent wells coated with PM-Scl antigen. In the first reaction step, diluted patient samples are incubated in the wells. If the sample is positive, specific IgG antibodies (also IgA and IgM) bind to the antigen. To detect the bound antibodies, a second incubation is carried out using an enzyme-labelled anti-human IgG (enzyme conjugate) which is capable of promoting 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	SAMPLEBUFFER
9. Wash buffer 10x concentrate	colourless	1 x 100 ml	WASHBUFFER 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. Protocol with target values	---	1 protocol	

LOT Lot description — CE — Storage temperature

IVD In vitro diagnostics — Unopened usable until

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 shoud be handled as infectious waste. All reagents should be disposed of according to official regulations.

Preparation and stability of the reagents

Note: All reagents must be brought to room temperature (+18°C to +25°C) around 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 a minimum of 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 crystallization 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 deionized 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 ready-to-use diluted wash buffer is stable for 1 month 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.

Warning: The calibrators and controls used have tested negative for HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 using enzyme immunoassays and indirect immunofluorescence methods. Nonetheless, all materials should be treated as being a potential infection hazard and should be handled with care. Some of the reagents contain the toxic agent sodium azide. Avoid contact with the skin.

Preparation and stability of the patient samples

Sample material: 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** sample buffer. For example: dilute 10 µl serum 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.

Sample incubation: (1. step)
Transfer 100 µl 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 400 µl 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 falsely low extinction values. Insufficient washing (e.g., less than 3 wash cycles, too small wash buffer volumes, or too short reaction times) can lead to falsely 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: (2. 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: (3. 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 of of 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.

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 reagent wells are break off format. Therefore, the number of tests performed can be matched to the number of samples, minimizing 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

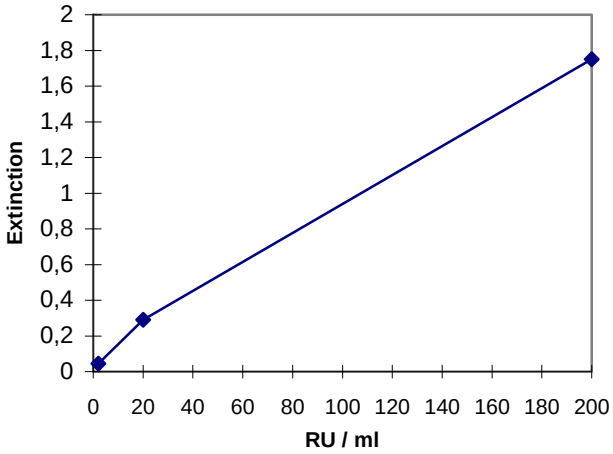
Semiquantitative: Results can be evaluated semiquantitatively by calculating a ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 2. Calculate the ratio according to the following formula:

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

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 of a patient sample lies above the value of calibrator 1 (200 RU/ml, the result should be given as ">200 RU/ml". It is recommended that the sample be re-tested at a dilution of 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 the sample should be retested.

For diagnosis, the clinical symptoms of the patient should always be taken into account alongside the serological results.

Test characteristics

Calibration: As no international reference serum exists for antibodies against PM-Scl, 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 ratios determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A protocol containing these target 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 activity of the enzyme used is temperature-dependent and the extinction values may vary if a thermostat is not used. The higher the room temperature (+18°C to +25°C) during substrate incubation, 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 a recombinant PM-Scl. The corresponding human cDNA was expressed in E. coli.

The PM-Scl antigen is a complex of 11-16 polypeptides with molecular weights of between 20 and 110 kDa. The main antigens are two polypeptides of 75 and 100 kDa, which are known as PM-Scl-75 and PM-Scl-100. 90-98% of PM-Scl autoantibody positive sera react with PM-Scl-100 and 50-63% with PM-Scl-75. The two antigens are independent of one another and do not show any cross reactivity. PM-Scl is mainly localized in the nucleoli, but also occurs in the nucleoplasm. The function of the polypeptide complex has not yet been fully explained. It is suspected that PM-Scl plays a role in splicing of the 5.85 rRNA and some U-snRNAs.

Linearity: The linearity of the test was investigated by assaying serial dilutions of patient sera with high antibody concentrations. The Anti-PM-Scl ELISA is linear in the measurement range 2 - 200 RU/ml.

Detection limit: The detection limit is defined as a value of three times the standard deviation of an analyte-free sample and is the smallest detectable antibody titer. The detection limit of the Anti-PM-Scl ELISA is approximately 1 RU/ml.

Cross reactivity: This ELISA showed no cross reactivity.

Interference: Haemolytic, lipaemic and icteric samples showed no influence at the result up to a concentration of 10 mg/ml for hemoglobin, 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 with values at different points on the calibration curve. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed on 6 different days.

Intra-Assay Variation, n = 20		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	48	3.4
2	129	2.6
3	168	1.7

Inter-Assay Variation, n = 4 x 6		
Serum	**Mean value (RU/ml)**	**CV (%)**
1	47	3.4
2	126	2.6
3	167	1.9

Specificity and sensitivity: 84 sera from patients with various inflammatory rheumatic diseases were examined with the EUROIMMUN Anti-PM-Scl ELISA (IgG) and the EUROIMMUN IIFT (IgG) as a reference method. Antibodies against PM-Scl produce a fluorescence in the nucleoli which can also be seen in the presence of antibodies against fibrillarin and RNA polymerases. A nucleolar staining pattern does not prove the presence of antibodies against PM-Scl but a negative IIFT result excludes their presence. The ELISA showed a specificity with respect to IIFT of 100%.

n = 84		**IFT**	
		positive	negative
ELISA	positive	21	0
	negative	10	53

23 sera from patients with an overlap of polymyositis and scleroderma were examined with the EUROIMMUN Anti-PM-Scl ELISA (IgG) and the EUROIMMUN HEp2 Westernblot (IgG). The test showed a sensitivity with respect to Westernblot of 92.9%.

Reference range: The levels of anti-PM-Scl antibodies (IgG) were analyzed with this EUROIMMUN ELISA in a panel of 136 healthy blood donors. With a cut-off of 20 RU/ml, all blood donors were anti-PM-Scl negative.

Clinical significance

Autoantibodies against PM-Scl were first described in 1977 by Wolfe and colleagues in patients with polymyositis and called anti-PM-1 antibodies. Further studies performed by Reichlin and colleagues in 1984 led to a more exact characterization of the specific PM-1 antibodies and to their naming (PM-Scl antibodies). They are detected in 50-70% of patients with so-called overlap syndrome, which combines symptoms of polymyositis (PM), dermatomyositis (DM), and progressive systemic sclerosis (Scl). The prevalence of PM-Scl autoantibodies amounts to 3% in progressive systemic sclerosis (diffuse form) and 8% in dermatomyositis and polymyositis.

In patients with anti-PM-Scl antibody positive sera, other autoantibodies are generally not detected, for example antibodies against extractable nuclear antigens (ENA) or against Jo-1 (histidyl-tRNA transferase). The absence of further autoantibodies such as anti-centromere autoantibodies or anti-Scl-70 autoantibodies in sera of anti-PM-Scl-positive patients shows that these patients have a disease entity between myositis and scleroderma. Among the clinical manifestations in patients with anti-PM-Scl autoantibodies are Raynaud's phenomenon (77-100%), arthritis and arthralgias (77-97%), myositis (50-95%), and interstitial lung diseases (32-88%).

As with most other autoimmune diseases, the etiology is unknown. A genetic predisposition is presumed, since PM-Scl autoantibodies are associated with HLA-DR3 (75-100% of patients). Women are affected more frequently than men.

The diagnosis of a PM-Scl autoantibody-associated overlap syndrome is of decisive importance for the therapy of affected patients. They generally require much lower doses of steroids (prednisone <7.5 mg/d) than patients with progressive systemic sclerosis (prednisone 20-30 mg/d, independent of the presence of myositis) and in most cases have a better prognosis.

PM-Scl autoantibodies persist in serum even after successful therapy with prednisone and remission of clinical symptoms. The antibody titer does not correlate with the seriousness of the disease.

Literature references

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