protagen DIAGNOSTICS

Multilisa® | Scl-70

REF 1010

Instruction For Use

Version 1.2

ELISA for the qualitative detection of autoantibodies against Scl-70 in human serum or plasma

Store kit components as recommended (2–8°C)

CE

Contents

1 Symbols used on labels

IVD	*In vitro* diagnostic medical device		Consult instructions for use
	Manufacturer		Keep away from sunlight
REF	Catalog number		Do not reuse
	Sufficient for 96 determinations		Date of manufacture
LOT	Batch number	CONTROL –	Negative control
	Use by	CONTROL +	Positive control
	Temperature limitations		

2 Intended use

The Protagen Multilisa® Scl-70 is an enzyme-linked immunosorbent assay (ELISA) for the qualitative determination of IgG anti-Scl-70 autoantibodies in human serum and plasma. The presence of anti-Scl-70 autoantibodies is used to aid in the diagnosis of Systemic Sclerosis, together with additional laboratory tests and clinical findings.

3 Scientific background

Systemic sclerosis (SSc) is a systemic autoimmune disease that manifests as progressive fibrosis of the skin and internal organs[1,2]. Circulating IgG autoantibodies targeted against Topoisomerase 1 / Scl-70 (ATA) are considered as being highly specific for the diagnosis of Systemic Sclerosis (SSc). ATA occur in 20–40% of systemic sclerosis patients. ATA are highly prevalent in patients with the diffuse form of SSc mostly accompanied with an increased risk of pulmonary fibrosis and digital ischemia (or digital ulcerations, DU). The existence of ATA is highly specific, and therefore were included in the ACR EULAR classification criteria for the diagnosis of SSc. Autoantibodies to Scl-70 exclude the serological diagnosis of other SARD than SSc. Serum levels of ATA do not fluctuate with the course of SSc.

4 Test principle

Recombinant human Scl-70 protein is bound to the bottom of ELISA microplates. The determination is based on an indirect enzyme linked 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 unspecific bound serum or plasma components. Enzyme conjugate is added and binds to the immobilized antibody-antigen complexes. After incubation, a second washing step removes unbound enzyme conjugate. After the addition of substrate solution, the bound enzyme conjugate hydrolyses the substrate forming a blue colored product. Addition of an acid stops 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 450nm. The test principle is shown in figure 1.

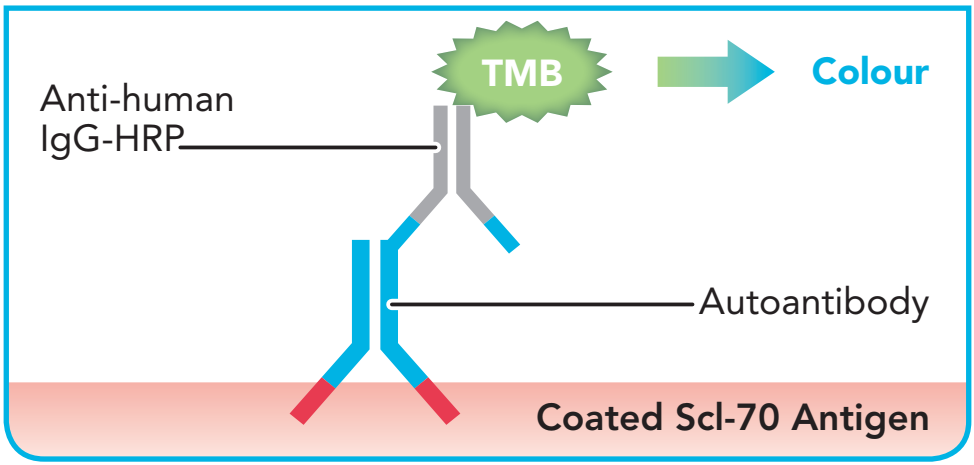


Figure 1. Recombinant human Scl-70 protein coated to ELISA microplates. Sample material: human Serum or EDTA plasma. The assay time is ~ 1.5 hours.

5 Kit content

Label	Content including function	Volume	Storage
Multilisa® Plate	Microtiter plate coated with recombinant human antigen. 12 modules of 8 wells, ready to use	1pc	2–8°C
CONTROL +	Positive control, red cap, red liquid, ready to use. Human serum/buffer matrix (PBS, BSA, NaN_3 0.09%, amaranth), negative for anti-HIV-AB, HBsAg, anti-HCV-AB	2ml	2–8°C
CALIBRATOR	Calibrator, yellow cap, yellow liquid, ready to use. Human serum/buffer matrix (PBS, BSA, NaN_3 0.09%, quinoline-yellow), negative for anti-HIV-AB, HBsAg, anti-HCV-AB	2ml	2–8°C
CONTROL –	Negative control, green cap, green, ready to use. Human serum/buffer matrix (PBS, BSA, NaN_3 0.09%, naphthol-green), negative for anti-HIV-AB, HBs-Ag, anti-HCV-AB	2ml	2–8°C
CONJUGATE	Anti-human IgG-HRP conjugate (MOPS, BSA, 0.02% methylisothiazolone, 0.02% bromonitrodioxane, 0.002% other active isothiazolones), yellowish, ready to use	15ml	2–8°C
DILBUF	Sample dilution buffer (HEPES, BSA, NaN_3 0.09%, Patent Blue V), ready to use	100ml	2–8°C
WASHBUF 10x	⚠ Wash buffer-concentrate; needs to be diluted before use 1 + 10 with double distilled water, colorless, foaming possible (contains PBS, salt, detergent)	100ml	2–8°C
SUBSTRATE	3,3',5,5'-tetramethylbenzidine (TMB), slightly blue, ready to use	15ml	2–8°C

5 Kit content (cont.)

Label	Content including function	Volume	Storage
STOP	⚠ 0.25 N H_2SO_4, ready to use	15ml	2–8°C
./.	Adhesive foil to cover microtiter plate	1pc	./.
	Instructions for use	1pc	./.
	Certificate of analysis	1pc	./.

CAUTION: The following reagents (as used in the assay and described in this instruction manual) are irritant and should be handled with care: Substrate (TMB Solution) and Stop Solution.

6 Additional material and equipment required

- Calibrated pipettes, multi-channel pipettes, single-channel multi-pipettes (with stepping mechanism for precise repeat dispensing) for volumes of 10µl, 100µl and 1000µl
- MTP ELISA reader, 450nm, optional reference filter at 620nm
- Double distilled water
- Laboratory timing device
- Measuring cylinder for 1000ml and 100ml
- Storage container for diluted wash buffer
- Data reduction software
- Optional MTP washer

7 Specimen collection, storage and handling

- Test is suitable for human serum or citrate plasma
- 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 stored at 2–8°C for up to five days or 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

8 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. However, please note, 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 VSV and BT virus and found negative.

⚠ Avoid contact with the substrate TMB (3,3´,5,5´-tetramethylbenzidine).

⚠ TMB substrate is light sensitive. Exposure of reagent to light during ELISA procedure should be limited (protect during incubation).

⚠ Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.

⚠ Control, sample buffer and wash buffer contain sodium azide 0.09% as a preservative. This concentration is classified as non-hazardous.

⚠ Enzyme conjugate contains 0.02% methylisothiazolone, 0.02% bromonitrodioxane, 0.002% other active isothiazolones as a 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 any assay 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 made of nitrile rubber or natural latex. Wear protective glasses. When used according to the instructions for use, there is no known hazard.

⚠ Conditions to avoid: Since substrate solution is light-sensitive, store in the dark.

⚠ For disposal of laboratory waste, please observe and follow any relevant national or regional legislation. Observe the guidelines for performing quality control in medical laboratories by assaying control sera.

⚠ Reagents and MTP modules of different lots **MUST NOT** be used in one test procedure.

⚠ For dilution purposes, only use double distilled water.

⚠ Bring all working solutions to ambient working temperature (18–24°C) before use.

⚠ **DO NOT** aliquot controls, conjugate or substrate.

⚠ Centrifuge controls and conjugate briefly before use and mix carefully; **DO NOT vortex!**

⚠ Pipet thoroughly to ensure accurate transfer of small volumes and avoid foaming.

⚠ Run controls and calibrator with each run.

⚠ Strictly perform the assay at the recommended incubation times and temperature.

⚠ Always use the same procedure to minimize inter-assay variance.

9 Preparation of reagents

1. Dilute the contents of 1 bottle of WASHBUF 10x concentrate in distilled or deionized water to a final volume of 1000ml prior to use.

⚠ Diluted wash buffer should be used within one week and can be stored in a closed plastic container at 4–8°C.

⚠ Samples have to be diluted in DILBUF.

⚠ Thaw samples at room temperature, mix thoroughly and spin down briefly.

2. Dilute the samples 1:101 in DILBUF. We recommend diluting 10µl of each sample with 1000µl DILBUF to get 1010µl.

⚠ Dilute samples prior to use. Do not store diluted samples longer than two working days.

⚠ Do not subject samples to repeated freeze-thaw cycles.

⚠ Controls and calibrator are ready to use and **MUST NOT** be diluted.

10 Assay procedure

⚠ All incubation steps have to be carried out at ambient working temperature (18–24°C)!

Step	Action	Volume/ well	Time
1	• Create plate layout. Testing calibrators and controls in duplicate is mandatory • Pipet diluted samples, calibrators and controls as indicated in the plate layout • Cover the MTP tightly with an adhesive cover foil • Incubate 30 min at ambient room temperature (18–24°C)	100µl	30 min
	• Remove samples and controls by aspirating and tapping • Wash 3 times with diluted WASHBUF • Remove the washing fluid by aspirating or tapping	3x 300µl	
2	• Pipet the anti-human IgG-HRP conjugate (ready to use) in all used wells • Cover the MTP tightly with an adhesive cover foil • Incubate 30 min at ambient room temperature (18–24°C)	100µl	30 min
	• Remove conjugate by aspirating and tapping • Wash 3 times with WASHBUF • Remove the washing fluid by aspirating or tapping	3x 300µl	
3	• Pipet SUBSTRATE (TMB solution) in all used wells • Cover the MTP tightly with an adhesive cover foil • Incubate 15 min at ambient room temperature (18–24°C) • Keep protected from direct light	100µl	15 min
	• Stop color development reaction by pipetting 50µl of STOP-solution in all wells – mix briefly	50µl	
	Photometric measurement: • Measure the optical density of each well at 450nm using a microplate reader within 15 min (you can subtract absorbance at a reference wavelength 620nm optionally)		

11 Validation

The assay is valid if

1. CONTROL + > 0.50 OD
2. CONTROL - < 0.30 OD
3. CALIBRATOR > 0.30 OD

⚠ If these quality control criteria are not met, the assay run is invalid and should be repeated.

12 Calculation of results

Calculate the score [AU] of the sample:

$$\text{Score}_{\text{sample}} = \frac{\text{OD}_{\text{Sample}}}{\text{OD}_{\text{Cal}}} \text{ x lot factor [AU]}$$

Score [AU]	Evaluation
< 0.9	Negative
0.9–1.1	Borderline
> 1.1	Positive

13 Expected results

A mean value for blood donors (n=88) was estimated with 0.122 OD or 0.3 AU.

⚠ Every laboratory should estimate its own reference values.

14 Validation data

- Intra-assay CV:

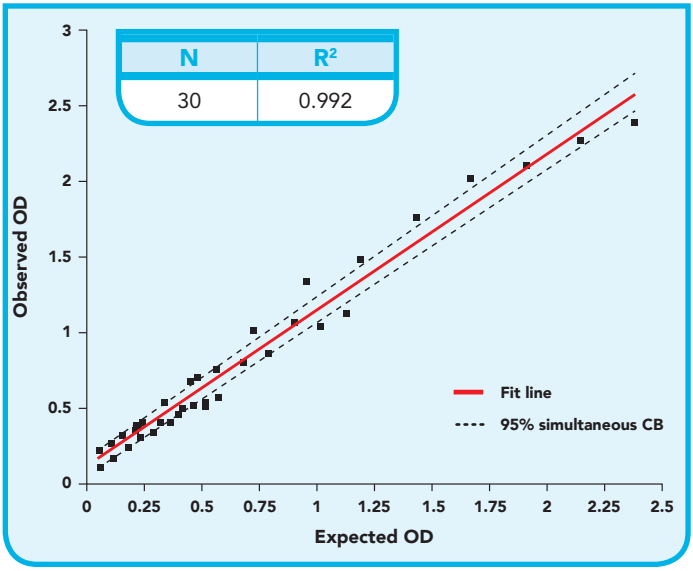
Intra-assay n=30, Unit=Index (sample/calibrator)	Mean AU	SD	95% CI	CV
Sample 1 (positive)	1.6	0.1	0.2	5%
Sample 2 (negative)	0.4	0.0	0.0	2%
Sample 3 (negative)	0.5	0.0	0.0	4%
Sample 4 (intermediate)	1.1	0.1	0.2	8%
Sample 5 (high positive)	3.1	0.1	0.2	2%

Excellent intra-assay variance of less than 10% was observed for 30-fold repeat of samples with different autoantibody titers against Scl-70.

- Linearity by dilution:

High linearity by dilution was observed with samples measured with the Multilisa® Scl-70.

Observed to expected results showed an excellent correlation coefficient of 0.992.



14 Validation data (cont.)

- Lot-to-lot reproducibility:

Repeatability (n=96)	Within Lot	Lot-to-Lot
Slope	0.9974	0.9286
	95% CI 0.9843 to 1.0017	95% CI 0.8571 to 1.0081
Intercept	1.0017	0.016
	95% CI 0.0082 to 0.0118	95% CI 0.0088 to 0.0250

Lot-to-lot consistency as shown on two independent kit lots of the Multilisa® Scl-70. Results show excellent repeatability within different lots of the assay.

- Interfering substances:

Interference substance (n=144)	Result
Hemoglobin	Up to 500mg/dl
Bilirubin	Up to 18mg/dl
Intralipid	Up to 1300mg/dl
Rheumatoid factor	Up to 500IU/ml

Multilisa® Scl-70 shows no significant influence of icteric, hemolytic and lipemic samples up to the specified concentration of the interferent.

15 Study results

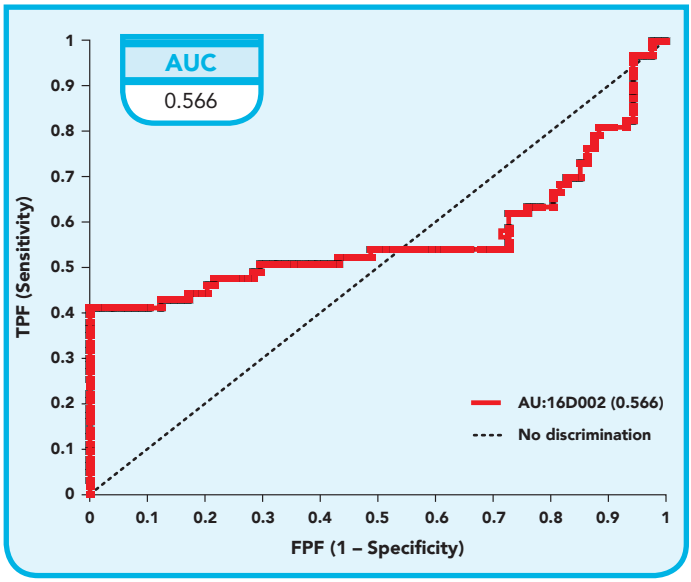
- In a recent clinical study the Multilisa® Scl-70 generated the following performance data:

TP proportion (sensitivity)	TN proportion (specificty)	Predictive value (+)	Predictive value (-)
0.406	1.000	1.000	0.700

Group	N=151
Healthy control	88
SSc	63

- Clinical sensitivity of 40.6%
- Clinical specificity of greater than 99%

15 Study results (cont.)



When comparing to the diagnosis of systemic sclerosis against healthy volunteer samples, ROC analysis revealed an area under the curve (AUC) value of 0.566.

16 Troubleshooting

This table describes various troubleshooting parameters.

Problem	Possible cause	Recommendation
Unexpected color development	Inadequate incubation time and temperature	Ensure that incubation intervals are correct and that all reagents reach 18–24°C before using in the test
	Contamination of water can negatively influence the test	Use double distilled water for reconstitution and preparation of the working solutions; take care that the water is not contaminated with microbes!
Questionable readings	Inappropriate filters in the MTP reader have been used	Ensure the filters in the MTP reader are the correct wavelength (450nm)
Weak or no signal	Sodium azide, ß-mercapthoethanol or DTT can interfere with the peroxidase reaction	Use only samples and solutions that do not contain sodium azide, ß-mercapthoethanol and DTT
Drift systematic bias	Unequal distribution of temperature in wells during incubation	Ensure that all reagents were brought to 18–24°C prior to use and use the recommended incubation times and temperatures
	Evaporation of fluids	Ensure adequate fixation of the adhesive cover foils during the incubation steps
Poor precision	Non-homogenous sample after freezing	Mix sample before pipetting
	Turbidity or particles in the sample	Mix sample well before pipetting, centrifuge sample to sediment particles
	Carry over between samples/controls	Change pipet tips between each pipetting step
	Unequal volumes added to the wells	Check pipette function and recalibrate if necessary
	Inadequate aspiration of fluids	No fluid should remain in the wells after aspiration
	Washing was incomplete	Ensure that the automatic washer is working properly

17 Literature

1. Tan EM: **Autoantibodies to Nuclear Antigens (ANA): their Immunobiology and Medicine**. *Adv Immunol* 1982, 33:167–240.

2. Pollard KM, Reimer G, Tan EM: **Autoantibodies in Scleroderma**. *Clin Exp Rheumatol*. 1989 Sep-Oct; 7 Suppl 3:S57–62.

18 Notes

18 Notes

18 Notes

protagen DIAGNOSTICS

19 Manufacturer

Protagen AG
Otto-Hahn-Str. 15
44227 Dortmund
Germany

T +49 231 97426300
F +49 231 97426301
E info@protagen.com
W protagen.com

Revision Date 2016/09

CE

Diagnostics differentiated.