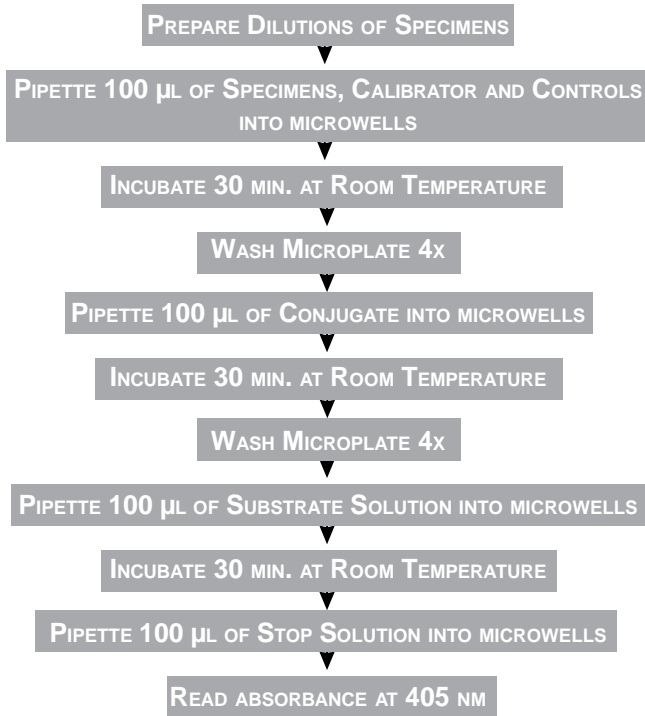


ImmulinTM PROCEDURE AT A GLANCE



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ImmulinTM Anti-Scl-70 Antibody ELISA

For Investigational Use Only

PRODUCT INSERT

Product Code: 1150

96 Determinations

INTENDED USE

An enzyme-linked immunosorbent assay (ELISA) for the detection and quantitation of antibodies to Scl-70 in human serum.

SUMMARY AND EXPLANATION

Antinuclear antibodies (ANA) occur in sera of patients with various connective tissue disorders such as *systemic lupus erythematosus* (SLE), mixed connective tissue disease (MCTD), *scleroderma*, *polymyositis* and Sjögren's syndrome. These ANA are directed to nuclear antigens, including DNA, nucleohistone and various extractable nuclear antigens (ENA) such as RNP, Sm, SS-A (Ro), SS-B (La), Scl-70 and Jo-1¹⁻⁹. These antigens are macromolecular complexes of protein and RNA. ENA are soluble ribonucleoprotein (snurps) complexes. Anti-Sm antibodies are disease specific and a marker for SLE. Antibodies to Sm occur in approximately 30-40% of SLE patients. They are rare in other systemic connective tissue diseases and if present, indicate either overlap of disease or patients that have not yet fulfilled the ARA criteria for SLE¹⁻⁹.

Other antibodies such as those directed against SS-A (Ro), SS-B (La) and RNP are not disease specific. Antibodies to RNP occur in 35-45% of SLE patients and in over 95% of patients with mixed connective tissue disease (MCTD). Occasionally they are also found in *scleroderma*, rheumatoid arthritis and drug induced LE. Antibodies to SS-A (Ro) and SS-B (La) occur in approximately 30-40% and 10-15% of SLE patients and 60-70% and 40-60% of patients with Sjögren's syndrome respectively. Antibodies to SS-B (La) occur frequently in association with SS-A (Ro) antibodies. Antibodies to Scl-70 are a specific serological marker for systemic scleroderma. Varying frequencies of Scl-70 antibodies have been reported. In early studies this antibody was detected in approximately 20% of patients with scleroderma¹⁰⁻¹² but later studies reported an incidence of 75% in patients with diffuse scleroderma and 44% in patients with acrosclerosis. These autoantibodies can be detected by various methods, such as gel-diffusion, Western Blot or ELISA.

PRINCIPLES OF PROCEDURES

The test is performed as a solid phase enzyme-labeled immunosorbent assay (ELISA). Microwells are coated with purified Scl-70 antigen. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows specific anti-Scl-70 antibodies present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Bound antibodies are detected by adding an enzyme labeled anti-human IgG conjugate to the wells. Unbound conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies is detected by a color change produced by the conversion of pNPP substrate to a colored reaction product.

The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. Results are expressed in ELISA Units per milliliter (EU/ml)

REAGENTS

Storage and Preparation

Store all reagents at 2-8°C. **Do not freeze.** Do not use if reagent is not clear or if a precipitate is present. All reagents must be brought to room temperature (20-25°C) prior to use. When stored at 2-8°C, the reconstituted wash buffer is stable until the kit expiration date. Reconstitute the wash buffer to 1 liter with distilled or deionized water. Coated microwells are for one time use only.

Precautions

For in vitro Diagnostic Use. All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. However, human blood derivatives and patient specimens should be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials³.

WARNING - Sodium azide (NaN₃) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources other than the same catalog number from IMMCO DIAGNOSTICS. Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling. Do not use beyond expiration date on the label.

Materials provided

ImmuLisa™ anti-Scl-70 antibody ELISA *Product Code: 1150*
Kit contains sufficient reagents to perform 96 determinations.

- | | |
|-------------------|---|
| 12 x 8 | Ready to use Microplate with individual breakaway microwells coated with Scl-70 antigen. |
| 1 x 1.5 ml | Ready to use Positive Control* (<i>red cap</i>). Contains human serum positive for Scl-70 antibodies. The expected concentration range in EU/ml is printed on the label. |
| 1 x 1.5 ml | Ready to use Negative Control* (<i>white cap</i>). Contains human serum. |
| 4 x 1.5 ml | Ready to use set of 4 Calibrators ; Calibrator A (<i>green cap</i>), Calibrator B (<i>violet cap</i>), Calibrator C (<i>blue cap</i>) and Calibrator D (<i>yellow cap</i>). Human serum containing antibodies to Scl-70 antigen. Concentration in EU/ml is printed on the label. |
| 1 x 12 ml | Ready to use anti-human IgG Alk. Phos. Conjugate* . Color coded pink. |
| 1 x 60 ml | Ready to use Serum Diluent* . Color coded blue. |
| 1 x 12 ml | Ready to use Enzyme Substrate* . Contains pNPP. Protect from light. |

LIMITATIONS OF THE PROCEDURE

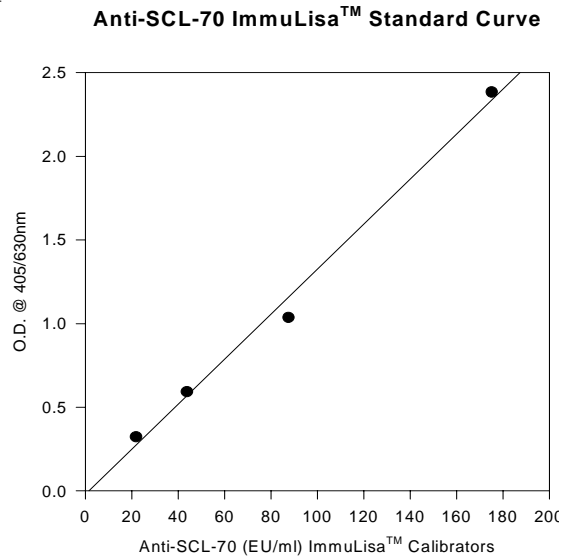
Results obtained with the ImmuLisa™ anti-Scl-70 assay serve only as an aid in the overall diagnosis and should not be interpreted as diagnostic in themselves.

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7. McCarty GA. Autoantibodies and their relation to rheumatic diseases. *Medical Clinics of North America*; 1986, 70:237-261.
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11. Jarzabek-Chozelska M et al. Scl-70 antibody - a specific marker of systemic sclerosis. *Br J Dermatol*; 1986, 115:393-401.
12. Kumar V, Beutner EH, Dabski K, Steger R and Koelle M. A standardized method of detecting antibodies to extractable nuclear antigens (RNP and Sm) by gel precipitation. *J Clin Lab Immunol*; 1984, 15:163-166.
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2. SEMI-QUANTITATIVE DETERMINATION

Plot absorbance of Calibrator A through D against their respective concentration on a linear-linear graph paper. Plot the concentration of EU/ml on the X-axis against the absorbance of the Y-axis and draw the best fit curve. Determine the concentrations of the patient samples from the curve against its corresponding absorbance value.



Calibrator

The Ready to Use Calibrators are included to provide semi-quantitation and must be included with each run. Patient samples containing higher antibody levels may give absorbance values greater than that of the Calibrator A. For determining accurate semi-quantitative values such serum samples should be further diluted so they fall within the range of the calibrator curve when retested. For determining EU/ml, multiply the units obtained by the dilution factor.

Interpretation

The following serves only as a guide in the interpretation of the laboratory results. Each laboratory must determine its own normal values. These may vary with the population examined. These may vary with the population examined.

anti-Scl-70 conc.	Interpretation
< 20 EU/ml	Negative
20-25 EU/ml	Indeterminate (Borderline)
>25 EU/ml	Positive

- 1 x 12 ml** Ready to use **Stop Solution**.
- 2 vials** Powder **Wash Buffer**. Reconstitute to one liter each.
- 1 x extra** Frame Holder
- 2 x** Protocol Sheets

*CAUTION - Contains <0.1% NaN₃

Materials Required But Not Provided

- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer of Automatic microplate washer capable of dispensing 200 µl
- Pipettes capable of delivering 5 µl to 1000 µl
- Disposable pipette tips
- Clean test tubes 12 x 75 mm and test tube rack
- Timer
- Absorbent paper towels
- Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm.

SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples.

PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let serum specimens and test reagents equilibrate at room temperature before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- All dilutions of the patient samples should be prepared prior to starting with the assay.
- Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. An automated microplate washer is recommended.
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
- Addition of all samples and reagents should be performed at the same rate and in the same sequence.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.

Test Method

- Step 1** Let all reagents equilibrate at room temperature.
- Step 2** Label protocol sheet to indicate sample placement in the wells. It is good laboratory practice to run samples in duplicate.
- Step 3** For a qualitative determination use only the Ready to Use Low Calibrator D (vial with yellow cap).
- or
For a **semi-quantitative determination** use the Ready to Use Calibrators A through D as depicted in the sample layout below.

QUALITATIVE DETERMINATION

A	BLANK	S5		
B	NEG	S6		
C	POS	S7		
D	CAL D	S8		
E	S1	S9		
F	S2	S10		
G	S3	S11		
H	S4	S12		
	1	2	3	4

SEMI-QUANTITATIVE DETERMINATION

A	BLANK	S2		
B	NEG	S3		
C	POS	S4		
D	CAL A	S5		
E	CAL B	S6		
F	CAL C	S7		
G	CAL D	S8		
H	S1	S9		
	1	2	3	4

- Step 4** Prepare a **1:101** dilution of patient specimen by pipetting **5 µl** of serum into **0.5 ml** of Serum Diluent. **Mix well.**
- Step 5** Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the microwells into the extra provided holder.
- Step 6** Pipette **100 µl** of Ready to Use Calibrator, Positive and Negative controls and patient samples to the appropriate microwells as per protocol sheet.
- Note:** Include one well which contains **100 µl** of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank. The absorbance of the reagent blank should not be more than 0.3 when read against air.
- Step 7** Incubate **30 minutes** (± 5 min) at room temperature.
- Step 8** Wash **4x** with the wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper. For automatic washers, program the washer as per manufacturer's instructions.
- Step 9** Pipette **100 µl** of Conjugate into microwells.
- Step 10** Incubate **30 minutes** (± 5 min) at room temperature.
- Step 11** Wash all microwells as in Step 7.

- Step 12** Pipette **100 µl** of Enzyme Substrate to each well in the same order and timing as for the conjugate.
- Step 13** Incubate **30 minutes** (± 5 min) at room temperature.
- Step 14** Pipette **100 µl** of Stop Solution into each microwell using the same order and timing as for the addition of the enzyme substrate. Read absorbance within 1 hour of adding Stop Solution.
- Step 15** Read absorbance of each microwell at **405 nm** using a single or 405/630nm dual wavelength microplate reader against the reagent blank set at zero absorbance.

Quality Control

Calibrator, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3 . The calibrator A should have an absorbance reading of not less than 1.0, otherwise the test must be repeated. The negative control must be <20 EU/ml. If the test is run in duplicate the mean of the two readings should be taken for determining EU/ml. While performing Qualitative determinations, the absorbance of the Calibrator D must be greater than that of the negative control and lesser than the absorbance of the positive control. For semi-quantitative determinations the positive control must give values in the range stated on the vial.

RESULTS

Calculations

The concentrations of the patient samples can be determined by either of two methods:

1. QUALITATIVE DETERMINATION

Abs. of Test Sample

Abs. of Calibrator D

X EU/ml of Calibrator D = EU/ml Test Sample