**ELISA kits available from ADI (see details at the web site)**

3300-100-SMG Human Anti-Smith antigen (Sm) IgG ELISA kit
3300-110-SRG Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit
3300-120-RNG Human Anti-RNP (RNP-70) IgG ELISA kit
3300-130-HNG Human Anti-histones IgG ELISA kit
3300-140-SCG Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit
3300-150-JOG Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit
3300-160-AFG Human Anti-Alpha Fodrin IgG ELISA kit
3300-170-CLG Human Anti-Cardiolipin IgG ELISA kit
3300-175-CLM Human Anti-Cardiolipin IgM ELISA kit
3300-185-CLA Human Anti-Cardiolipin IgA ELISA kit
3300-190-B2G Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit
3300-195-B2M Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit
3300-200-B2A Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit
3300-205-APS Human Anti-Phospholipid Screen (anti-Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and beta-2-Glycoprotein I) IgG/IgM ELISA kit
3300-210-PSS Human Anti-Phosphotidyl serine IgG/IgM ELISA kit
3300-215-PIS Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit
3300-220-PAS Human Anti-Phosphotidic Acid IgG/IgM ELISA kit
3300-230-APG Human Anti-Prothrombin IgG/IgM ELISA kit
3300-235-APA Human Anti-Prothrombin IgA ELISA kit
3300-240-AVA Human Anti-Annexin V IgG ELISA kit
3300-250-ANG Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit
3300-255-PRG Human ANCA (Anti-PR3) IgG ELISA kit
3300-265-MPG Human ANCA (Anti-MPO) IgG ELISA kit
3300-270-GBG Human Anti-glomerular basement membrane (GBM) IgG ELISA kit
3300-280-BPG Human Anti-bactericidal permeability increasing (BPI) protein IgG ELISA kit
3300-290-ELG Human Anti-Elastase IgG ELISA kit
3300-300-GLG Human Anti-Gliadin IgG ELISA kit
3300-305-GLM Human Anti-Gliadin IgM ELISA kit
3300-310-GLA Human Anti-Gliadin IgA ELISA kit
3300-315-PRG Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell (H//K/ATPase) IgG ELISA kit
3300-320-ASC Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG ELISA kit
3300-330-ASG Human Anti-Sperm IgG ELISA kit
3300-340-CCG Human Anti-Cyclic Citrullinated Peptide (CCP)IgG ELISA kit
3300-350-TPG Human Anti-thyroid peroxidase (TPO) IgG ELISA kit
3300-360-TGG Human Anti-thyroglobulin (TG) IgG ELISA kit

**#3000** Human Rheumatoid Factors IgM (RF)
**#3100** Human anti-dsDNA
**#3200** Anti-Nuclear Antibodies (ANA)

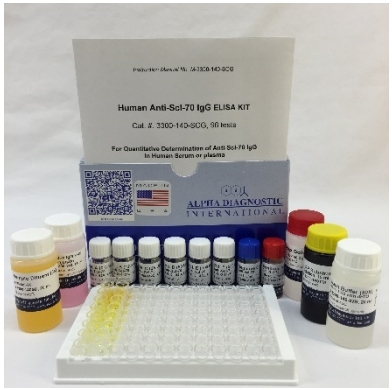


*Instruction Manual No. M*-3300-140-SCG

# Human Anti-Scl-70 IgG ELISA KIT

Cat. #. 3300-140-SCG, 96 tests

## For Quantitative Determination of Anti-Scl-70 IgG In Human Serum or plasma




***For In Vitro Research Use Only (RUO)***

ALPHA DIAGNOSTIC INTERNATIONAL

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Your Molecular & Cell Technology Partner

**DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.**

**Human Anti-Scl-70 IgG ELISA KIT**

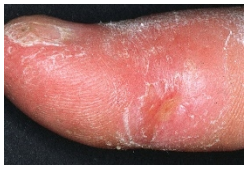
Kit Contents: Cat. 3300-140-SCG (reagents for 96 tests)

| Kit Components | |
|---|---|
| Purified Scl-70 coated **ELISA strips** (96 wells); #3300-141 | 1 plate |
| **Calibrator A**, (0 U/ml), 1.5 ml; #3300-142A, ready to use | 1 vial |
| **Calibrator B**, (12.5 U/ml), 1.5 ml; #3300-142B, ready to use | 1 vial |
| **Calibrator C**, (25 U/ml), 1.5 ml; #3300-142C, ready to use | 1 vial |
| **Calibrator D**, (50 U/ml), 1.5 ml; #3300-142D, ready to use | 1 vial |
| **Calibrator E**, (100 U/ml), 1.5 ml; #3300-142E, ready to use | 1 vial |
| **Calibrator F**, (200 U/ml), 1.5 ml; #3300-142F, ready to use | 1 vial |
| Anti-Scl-70 **Positive control**, 1.5 ml, # 3300-142P, ready to use | 1 vial |
| Anti-Scl-70 **Negative control**, 1.5 ml, # 3300-142N, ready to use | 1 vial |
| **Sample Diluent (5X)**, 20 ml (Yellow color) #3300-140SD | 1 bottle |
| Anti-h IgG **HRP Conjugate**, 15 ml, # 3300-143 | 1 bottle |
| **HRP Substrate** Solution (TMB) , 15 ml, # 3300-140TM | 1 bottle |
| **Stop Solution** (diluted HCl acid), 15 ml, # 3300-140ST | 1 bottle |
| **Wash buffer (50X),** 20 ml, # 3300-140WB | 1 bottle |
| Complete Instruction Manual, M-3300-140-SCG | 1 |

## Intended Use:

ADI's Anti-Scl-70 is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against Scl-70 in human serum or plasma. The kit is intended for in vitro research use only (RUO) as an aid in the diagnosis of scleroderma.

## INTRODUCTION



Rheumatoid autoimmune diseases are often associated with the occurrence of autoanti-bodies against several nuclear or cytoplasmic antigens. Today the best investigated immunoreactive antigens are double-stranded DNA (dsDNA), single stranded DNA (ssDNA), Sm (Smith), sn-RNP (small nuclear ribonucleoprotein particles), the complex RNP/Sm which is stabilized by ribonuleic acid as well as SS-A (Ro) and SS-B (La). The antigen Scl 70, a 70 kD molecular weight protein, also known as DNA-topoisomerase-1, is associated with scleroderma. Anti Scl-70 antibodies (also called anti-topoisomerase I) is a type of anti-nuclear autoantibody seen mainly in diffuse systemic scleroderma, but is also seen the more limited form of systemic scleroderma called CREST syndrome. Anti Scl-70 antibodies are associated with more severe scleroderma disease.

Today the best investigated immunoreactive antigens are double-stranded DNA (dsDNA), single stranded DNA (ssDNA), Sm (Smith), sn-RNP (small nuclear ribonucleoprotein particles), the complex RNP/Sm which is stabilized by ribonuleic acid as well as SS-A (Ro) and SS-B (La). The antigen Scl 70, a 70 kD molecular weight protein, also known as DNA-topoisomerase-1, is associated with scleroderma. In rheumatoid autoimmune diseases various profiles of autoantibodies to these antigens can be detected. In a high incidence they are related to active and inactive systemic Lupus erythrematodes, mixed connective tissue diseases (Sharp Syndrome), rheumatoid arthritis, Sjögren-Syndrome, Scleroderma, photosensitve dermatitis and drug-induced lupus. In Lupus patients typically anti-dsDNA antibodies can be detected. Patients without these antibodies very often show anti-ssDNA antibodies and anti-SS-A and anti-SS-B are present. A strong correlation between antibody concentration and severety of the disease has been observed with higher antibody concentrations in active phases of the disease. Thus quantitation is more informative compared to simple titering by immunoflourescence. Most of these parameters are not specific for just one disease but they occur in various combinations. The pattern of different antibody combinations and their concentration together with the whole clinical picture of the patient are helpful diagnostic tools in the assessment of rheumatoid autoimmune diseases. Anti-Scl-70 is found in ~75% patients with the diffuse progressive form of scleroderma. The presence of anti-Scl-70 seems to exclude the presence of anti-centromere antibody, which is a marker for a subset of patients with scleroderma known as CREST (calcinosis, sclerodactyly, and telangiectasia) syndrome.



## *Intra-assay precision:*

Three serum samples (Abs. ) were run 24 independent assays. The samples showed good intra-assay precision ( % CV).

| Sample No | Mean (U/ml) | CV (%) |
|---|---|---|
| 1 | 45.7 | 4.0 |
| 2 | 90.4 | 3.2 |
| 3 | 184.1 | 3.4 |

## *Inter-assay precision:*

| Sample No | Mean (U/ml) | CV (%) |
|---|---|---|
| 1 | 41.1 | 2.8 |
| 2 | 89.9 | 2.8 |
| 3 | 157.4 | 2.3 |

## LINEARITY

Three different samples were diluted (1:100, 1:200, and 1:400) and their Anti-Scl-70 levels determined. The samples showed excellent mean recoveries of about 102% (range %).

## SPECIFICITY

The microplate is coated with Scl-70 antigen highly purified by affinity chromatography. The Anti-Scl-70 test kit is specific only for autoantibodies directed to Scl-70. No cross reactivity to the other ENA-antigens have been observed.

## Sensitivity

The lower detection limit for Anti-Scl-70 has been determined at 1.0 U/ml.

## Species Reactivity

This kit is designed to test human samples. It detects IgG class of anti-Scl70 antibodies with no or minimal detection of IgG, IgA or IgE antibodies.

ADI has separate kits to detect slc70 antibodies in monkey, mouse or rat samples.

## INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not met, the results are invalid and the test should be repeated.

## Calculation of results

For Anti-Scl-70 a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice. Smoothed Spline Approximation and log-log coordinates are also suitable.

## Recommended Lin-Log Plot

First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

## Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-Scl-70 test:

| | Anti-Scl-70 [U/ml] |
|---|---|
| normal: | < 15 |
| borderline: | 15 - 25 |
| elevated: | > 25 |

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-Scl-70 antibodies. The above reference ranges should be regarded as guidelines only.



## PRINCIPLE OF THE TEST

Anti-Scl-70 ELISA kit is based on binding of Anti-Scl-70 from serum samples to scl70 antigen immobilized on microtiter wells. After a washing step, rabbit anti-human IgG-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (blue color) is directly proportional to the amount of Anti-Scl-70 present in the sample. The reaction is terminated by adding stopping solution (converts blue to yellow). Absorbance is then measured on a microtiter well ELISA reader at 450 nm. The concentration of Anti-Scl-70 in samples and control is read off the standard curve.

## MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-100 µl) and multichannel pipet; Reagent troughs, plate washer (recommended) and ELISA plate Reader.

## PRECAUTIONS

This ELISA Kit is intended for *in vitro research* use only. The reagents contain thimerosal (0.02%); necessary care should be taken when disposing solutions.

Applicable **SDS**/**MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site. TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).
http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

## SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed to clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed, these could be stored at -20$^{o}$C for up to six months. Avoid repeated freezing and thawing of samples.

## REAGENT PREPARATION FOR THE ASSAY

**Sample buffer :** Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use (or 1 ml stock in 4 ml water). Store refrigerated: stable at 2-8°C for at least 30 days

***Wash Buffer Concentrate*** *(50X solution).* Before use, dilute 1:50 with distilled water (20 ml stock bottle in 1-Liter water). Occasionally, some salts may form crystals during storage in cold but they redissolve upon slight warming of the solution.

**Sample preparation:** Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well. Controls are ready to use and need not be diluted.

**NOTES: Read instructions carefully before the assay**. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is

important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. Addition of the HRP substrate starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence.

## STORAGE AND STABILITY

All kit components are stable at 2-8$^{O}$C until the expiration date printed on the label. The whole kit stability is at least 6 months from the date of manufacture under appropriate storage conditions. The unused strips should be stored tightly covered with adhesive film and with the desiccant in the bag.

**TEST PROCEDURE** *(ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE)..*

1. Label, and secure the microtiter well strips to be used on the plate. **Dilute wash buffer(50X),** sample buffer(5X) & dilute patients samples 1:100 with sample buffer, before use.
2. Pipet **100 µl** of calibrators, controls & prediluted samples into appropriate wells in *duplicate*. Mix gently, cover the plate and incubate for **30 minutes** at room temp.(20-28 oC).
3. Aspirate and **wash** the wells **3 times** with 300 µl of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **100 µl** of enzyme conjugate into each well**.** Mix gently. Cover the plate and incubate for **15 minutes** at room temp.
5. Aspirate and **wash** the wells **3 times** as above.
5. Add **100 µl** of TMB Substrate into each well. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature.
6. **Stop** the reaction by adding **100 µl** of stop solution to all wells. Mix gently. Measure the absorbance at 450 nm using an ELISA reader (The color is stable for at least 30 min). Wells with lowest color may become clearer because of color fading with time.

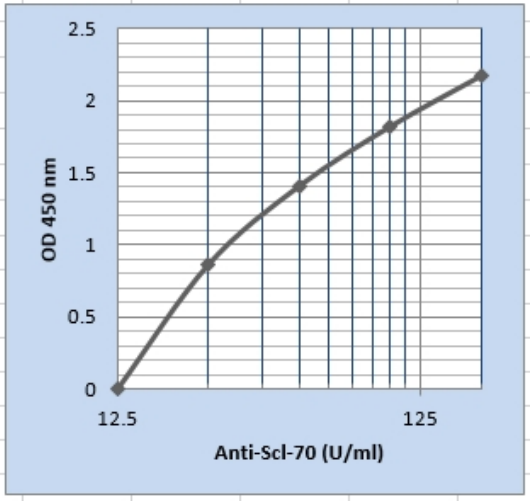


## WORKSHEET OF TYPICAL ASSAY

| Wells | Stds/samples U/ml | *Mean A450 nm | Calculated Concn. |
|---|---|---|---|
| A1, A2 | **Std A** (0) | 0.065 | |
| B1, B2 | **Std B (12.5)** | 0.633 | |
| C1, C2 | **Std C (25)** | 0.966 | |
| D1, D2 | **Std D (50)** | 1.411 | |
| E1, E2 | **Std E (100)** | 1.824 | |
| F1, F2 | **Std F (200)** | 2.176 | |
| G1, G2 | **Sample 1** | | |

***=** Average duplicate values after deducting the std zero values.

**NOTE:** These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.




6-ADI-ELISA-Arif-graph

A typical std. Assay (**do not use this for calculating sample values**)