



User's Manual

Anti-Sci-70 ELISA Kit

REF DEIA107J

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

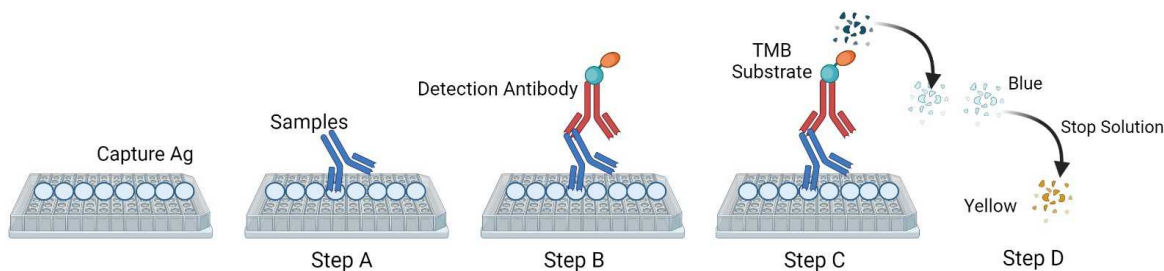
Anti-Scl-70 ELISA kit measures anti-Scl-70 antibodies in the serum.

General Description

Antibodies to Scl-70 are a specific immunological marker for scleroderma (or progressive systemic sclerosis, PSS), a systemic autoimmune disease characterized by collagen deposition and connective tissue destruction of the skin, blood vessels and certain internal organs. Studies have shown varying frequencies of Scl-70 antibodies in PSS. This antibody was found in approximately 20% of PSS patients in early studies but 75% in later studies. Scl-70 antibodies are directed against DNA-topoisomerase I which locates in the nucleus. The whole molecule of DNA topoisomerase is 110 kDa but it is easily degraded by proteases to 100 kDa, 87 kDa and 70 kDa (Scl-70). PSS is classified into two types; diffuse scleroderma and limited scleroderma. Scl-70 antibodies are present specifically in diffuse scleroderma and centromere antibodies are present in limited scleroderma. Rarely, Scl-70 antibodies are found in SLE and MCTD patients.

Principles of Testing

Anti-Scl-70 ELISA kit measures anti-Scl-70 antibodies in the serum. It is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes Scl70 protein for immobilization on the microtiter wells and anti-human IgG antibodies conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two components, resulting in anti-Scl-70 antibodies being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of anti-Scl-70 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Reagents And Materials Provided

1. 8x12 96-well plate coated with Scl-70.
2. Anti-human IgG antibody conjugated to HRP.
3. 1x Diluent buffer.
4. 5x Assay wash buffer.
5. Substrate.

6. Stop Solution.

Materials Required But Not Supplied

1. Microplate reader capable of measuring absorbance at 450 nm
2. Shaker

Storage

1. Store all reagents at 2-8°C.
2. All reagents must be brought to room temperature (20-25°C) prior to use.
3. When stored at 2-8°C, the diluted Assay wash buffer is stable until the kit expiration date.

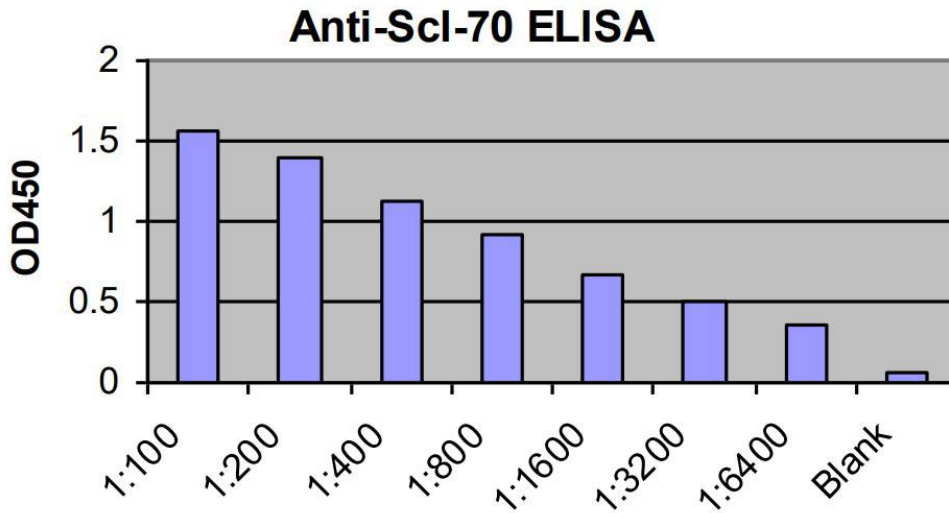
Reagent Preparation

1. Dilute the **5x Assay wash buffer** to **1x buffer**: 40 ml 5x Assay wash buffer and 160 ml ddH₂O
2. Dilute **1000 times of anti-human IgG antibody conjugated** with 1x Diluent buffer.

Assay Procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed.
2. Add 10 diluted samples (1:100 diluted or further 2 serial diluted serum) per well and incubate for 1 hour at room temperature with gentle shaking.
3. Aspirate each well and wash by adding 200µl of 1x Assay wash buffer. Repeat the process twice for a total of three washes. Completely remove liquid at each wash by firmly tapping the plate against clean paper towels.
4. Add 100µl of diluted anti-human IgG antibody conjugated to HRP to each well and incubate for 0.5 hours at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100µl of Substrate to each well and incubate for 5-30 minutes.
7. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
8. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Typical Standard Curve



Precautions

Human blood derivatives and patient specimens should be considered potentially infectious. All human derived components need to be tested for the negative HBsAg, HCV, HIV-1 and 2 and HTLV-I. Follow good laboratory practices in storing, dispensing and disposing of these materials.