Signosis
Innovative Plate Assay Solutions

Anti-Scl-70 ELISA Kit

Catalog Number EA-5007 **(For Research Use Only)**

Introduction

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.

Principle of the assay

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 Scl-70 protein for immobilization on the microtiter wells and anti-mouse 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.

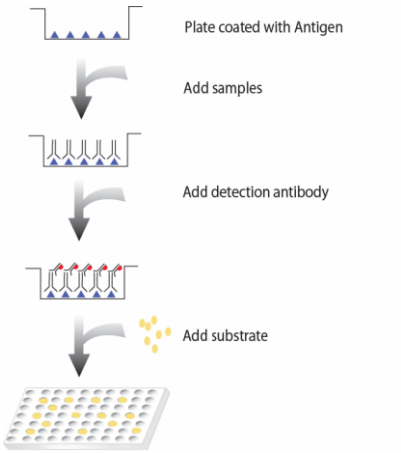

Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
8x12 96-well strip Plate coated with Scl-70	1	4°C
Anti-Human IgG antibody conjugated to HRP	10µL	4°C
Positive control	10µL	-20°C
1xDiluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	4°C

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Shaker

Reagent preparation before starting experiment

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

Storage and Preparation

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

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.

Assay procedure

1. Take the desired number of well strips from the plate. Make sure the rest of strips are well sealed.
2.Add 100 µl of diluted samples or positive control (1:100 diluted with 1X Diluent Buffer) per well and incubate for 1 hour at room temperature with gentle shaking. *Note: We recommend having a blank condition. For the blank, add only 1x Diluent buffer to the well.
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 30 minutes 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 7-30 minutes. *Note: Positive control will turn blue. Samples should be stopped when blue color begins to appear in blank.
8. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
9. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.