



User's Manual

Anti-SSA (Ro-60) ELISA Kit

REF

DEIA103J



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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.

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PRODUCT INFORMATION

Intended Use

Enzyme immunoassay for determination of Ro-60.

General Description

Antibodies to the SSA antigen (also known as Ro antigen) are one of the most frequent serological markers of autoimmunity in rheumatic diseases. They appear in 60-70% of patients with Sjögren's syndrome (SS), 30-40% of patients with systemic lupus erythematosus (SLE), and 3-5% of patients with rheumatoid arthritis (RA). The SSA antigen is comprised of an acidic 60 kDa protein that may exist in complex with RNA (80-112 bases). However, RNA is not required for SSA antigenicity. The SSA antigen is predominantly cytoplasmic, and not located in the nucleus. Therefore, patients with antibody to SSA may be ANA negative on routine testing. Approximately 62% of ANA negative lupus patients have antibodies to SSA. Two types of anti-Ro/SSA antibodies have been identified; Ro/SSA antigens of 60 kDa and 52 kDa. Anti-SSA-60 kDa antibodies are linked to certain disorders such as SS, SLE, neonatal lupus and congenital heart block. Clinically, the presence of aSSA52 has been reported in a wide variety of diseases, includes inflammatory myositis, primary biliary cirrhosis and SS.

Principles of Testing

Anti-SSA ELISA kit measures anti-SSA antibodies in the serum. It is based on the principle of a solid phase enzymelinked immunosorbent assay. The assay utilizes SSA 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-SSA 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-SSA is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

Reagents And Materials Provided

1. 8×12 96-well strip Plate coated with SSA (Ro-60), 1, 4°C
2. Anti-Human IgG antibody conjugated to HRP, 10µL, 4°C
3. 1× Diluent buffer, 40mL, 4°C
4. 5× Assay wash buffer, 40mL, 4°C
5. Substrate, 10mL, 4°C
6. Stop solution, 5mL, 4°C

Materials Required But Not Supplied

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

Storage

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.

Reagent Preparation

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

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 (1:100 diluted with 1× 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 1× 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. 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.

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.

