



SSA IgG ELISA Kit

(Catalog # E4979-100; 96 assay; Store at 4°C)

I. Introduction:

Sjogren syndrome (SS)/Ro are autoantigens that are a type of extractable nuclear antigen (ENA). SSA consists of 2 proteins: Ro60 and Ro52. Ro60 binds to uncoded RNA to form an hY-RNA complex and thereby inhibits the immune response. Ro52 is a protein formed either due to viral infection, type I interferons, or Toll-like receptors. Antibodies to SSA/Ro antigen are detected in patients suffering from autoimmune diseases such as systemic lupus erythematosus (SLE), Sjögren's syndrome (SS), systemic sclerosis, and rheumatoid arthritis (RA). BioVision's SSA IgG (Human) ELISA kit quantitatively measures SSA IgG antibodies in human serum and plasma samples. Samples, calibrator, and controls are added to the wells pre-coated with SSA antigen. IgG antibodies to SSA, if present in the samples, will bind to the SSA antigen. The wells are then washed with Wash Buffer, followed by incubation with the enzyme conjugate. After incubation, any unattached conjugates are washed off by Wash Buffer. The enzymatic reaction is detected by the addition of TMB-substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is directly proportional to the amount of IgG antibodies to SSA present in the sample

II. Features and Benefits:

Easy, convenient and time-saving method to measure the amount of SSA IgG in serum and plasma samples
No significant cross-reactivity or interference with other autoantibodies was observed

III. Sample Type:

Serum, Plasma

IV. Kit Contents:

| Components | E4979-100 | Part Number |
|---|---------------|-------------|
| Micro ELISA plate coated with SSA antigen | 8 x 12 Strips | E4979-100-1 |
| Sample Diluent | 22 ml | E4979-100-2 |
| Calibrator | 1 ml | E4979-100-3 |
| Positive Control | 1 ml | E4979-100-4 |
| Negative Control | 1 ml | E4979-100-5 |
| Enzyme Conjugate | 12 ml | E4979-100-6 |
| TMB Substrate | 12 ml | E4979-100-7 |
| Stop Solution | 12 ml | E4979-100-8 |
| Wash Buffer (20X) | 25 ml | E4979-100-9 |

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Distilled or deionized water

VI. Storage Conditions and Reagent Preparation:

- The entire kit may be stored at 4°C.
- **Wash buffer (1X)**: Prepare **Wash buffer (1X)** by adding 25 ml of **Wash Buffer (20X)** to 475 ml of distilled or deionized water. Store at room temperature (20-25 °C).
- Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Samples may be refrigerated at 2–8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing. Lipemic or hemolyzed samples may cause erroneous results.

VII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Place the desired number of coated strips into the holder.
2. Negative Control, Positive Control, and Calibrator are ready to use. Prepare 1:21 dilution of test samples, by adding 10 µl of the sample to 200 µl of **Sample Diluent** and mix well.
3. Dispense 100 µl of **diluted serum, Calibrator and Controls** into the appropriate wells. Add 100 µl **Sample Diluent** in well for black control. Tap the plate to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Aspirate liquid from all wells. Wash wells three times with 300 µl of **Wash Buffer (1X)**. Blot on absorbance paper or paper towel.
5. Dispense 100 µl of **Enzyme Conjugate** to each well and incubate for 20 minutes at room temperature.
6. Remove **Enzyme Conjugate** from all wells. Wash wells three times with 300 µl of **Wash Buffer (1X)**. Blot on absorbance paper or paper towel.
7. Dispense 100 µl of **TMB substrate** and incubate for 10 minutes at room temperature.
8. Add 100 µl of **stop solution**.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

VIII. Calculation:

Check **Calibrator Factor (CF)** value on the calibrator bottle. This value might vary from lot to lot.



Calculate the cut-off value: **Calibrator OD x Calibrator Factor (CF)**.

Calculate the **Ab (Antibody) Index** of each determination by dividing the O.D. value of each sample by cut-off value.

IX. Related Products:

SSB IgG (Human) ELISA Kit (E4978)

MOG (Human) ELISA Kit (E4963)

Complement C3 (Human) ELISA Kit (E4976)

AIRE (Human) ELISA Kit (E4971)

Muellerian-inhibiting Factor (AMH)(Human) ELISA Kit (E4538)

FOR RESEARCH USE ONLY! Not to be used on humans.