anti-SSA ELISA KIT

Cat. No.: DEIA05729
Pkg. Size: 96T

Intended use

For the detection and semi-quantitation of antibodies against the SSA (Ro) antigen in serum as an aid in the diagnosis of autoimmune disease.

General Description

Systemic rheumatic disease is characterized by the presence of circulating autoantibodies that are widely reactive with both nuclear and cytoplasmic antigens. Antibodies to SSA (Ro) are present in approximately 60 to 70% of patients with Sjogren’s Syndrome and 30 to 40% of patients with systemic lupus erythematosus (SLE). In addition, the presence of antibody to the SSA (Ro) antigen strongly correlates with distinguishable subsets of SLE and Sjogren’s Syndrome. Antibody to SSA (Ro) occurs in about 60% of patients with “ANA Negative” SLE, 63% of patients with subacute cutaneous erythematosus and in 75% of the homozygous C2-deficient patients with an SLE-like presentation. The existence of this autoantibody in pregnant women has been closely associated with the development of neonatal congenital heart block and neonatal lupus. Until recently, many laboratories used Immunodiffusion (ID) to detect anti-SSA antibodies. However, ID is time-consuming to perform and is relatively insensitive compared to newer methods. Enzyme immunoassay (EIA) has advantages over the ID method in sensitivity, simplicity, ease of automation and testing turnaround time.

The anti-SSA Test Kit is an EIA procedure intended for the semi-quantitation of antibodies to SSA (Ro) antigen. The results are reported in ELISA units (EU) per ml determined by comparison to a Calibrator.

Principle Of The Test

Purified SSA (Ro) antigen from bovine spleen and/or thymus is bound to microwells. Diluted patient sera, Calibrator, and controls are placed in the microwells and incubated. Anti-SSA (Ro) antibodies, if present, will bind to the antigen in the microwells. After washing the microwells to remove unbound antibodies, a second incubation with anti-human IgG conjugated to alkaline phosphatase is carried out. The conjugate will bind to human anti-SSA (Ro) antibodies, if present, forming an immunocomplex. The microwells are then washed again to remove unbound components and the enzyme substrate, para-nitrophenylphosphate is added. The enzyme, if bound, will catalyze the hydrolysis of the substrate to para-nitrophenol and result in formation of a yellow color. The reaction is then stopped and the color read with a photometer at 405 nm (reference at 600-630nm). The intensity of the color developed is proportional to the concentration of anti-SSA (Ro) IgG present in the sample.

Reagents And Materials Provided

Each anti-SSA Kit contains reagents for 96 tests.

1. **Antigen Wells** Twelve, 8-well microwell breakapart strips, color coded yellow, coated with SSA antigen.
2. **Calibrator** One vial containing 0.25 ml of human serum, 0.1% sodium azide. Assigned value printed on label.
3. **Negative Control** One vial containing 0.25 ml of non-reactive human serum, 0.1% sodium azide.
4. **Positive Control** One vial containing 0.25 ml of reactive human serum, 0.1% sodium azide. Assigned range printed on label.
5. **Sample Diluent** One bottle containing 60 ml Phosphate buffer with protein stabilizers. Contains Proclin 300, 15 ppm active ingredient. Color coded blue.
6. **Wash Concentrate (20X)** Two bottles containing 50 ml of Phosphate buffer with detergent and Proclin 300, 15 ppm active
ingredient. Each bottle is sufficient to make 1050 ml of wash solution.

7. **Conjugate** One bottle containing 25 ml goat anti-human immunoglobulin G labeled with alkaline phosphatase. Also includes protein stabilizers and Proclin 300, 30 ppm active ingredient. Color coded pink.

8. **Substrate** One bottle containing 25 ml para-Nitrophenyl phosphate in a buffered solution. Substrate solution may develop a slight yellow color upon storage.

9. **Stop Solution** One bottle containing 25 ml Sodium phosphate, tribasic. **CAUTION:** Solution is caustic. Avoid contact with skin. If contact is made, flush area with copious amounts of water.

**Store these reagents at 2 to 8°C.**

**Materials Required But Not Supplied**

**Manual Users:**
- 1. Wash bottle or automated microplate washer.
- 2. Pipettors capable of dispensing appropriate volumes.
- 3. Timer.
- 4. One liter graduated cylinder.
- 5. One liter wash solution reservoir.
- 6. Deionized or distilled water.
- 7. Absorbent toweling.
- 8. Tubes or microwell plate for sample dilution.
- 9. Reader capable of reading absorbance at 405 nm, reference at 600-630 nm

**Automated EIA Processor Users:**
- 1. One liter graduated cylinder.
- 2. Deionized or distilled water.
- 3. Pre-dilution cups, strips or plates.
- 4. ProbeClean Concentrate, or tip washing detergent solution, if applicable.

**Specimen Collection And Handling**

Whole blood should be collected by accepted medical techniques. The serum is separated from the clot and refrigerated at 2 to 8°C for short term storage (up to 7 days), or stored frozen at –20°C for long term storage. Avoid multiple freeze- thaw cycles. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Grossly contaminated, hemolyzed, lipemic, or icteric specimens should not be used.

**CAUTION:** Serum samples must not be heat-inactivated prior to use.

**Assay Steps**

**MANUAL USERS:**
1. Prepare 1:101 dilutions of the calibrator, controls, and patient samples in Sample Diluent. (e.g., by addition of 2 µl sample to 200 µl Sample Diluent or 5 µl sample to 500 µl Sample Diluent).
2. Mix sample dilutions gently by withdrawing and expelling in a pipette tip 2 or 3 times or by vortex mixing for 2 or 3 seconds. Transfer 100 µl of diluted calibrator, control, or patient sample, to the antigen wells. Avoid formation of bubbles when transferring diluted samples.
3. Allow the wells to incubate at room temperature (18 - 30°C) for 30±5 minutes.
4. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling if
necessary. Wash the wells by rinsing 3 times with at least 300 µl per well of Wash Solution. Remove excess moisture from the wells after washing. When using an automated washer, follow the manufacturer’s instructions.
5. Place 100 µl of Conjugate into each well, avoiding bubble formation.
6. Allow the wells to incubate uncovered at room temperature (18 - 30°C) for 30 ± 5 minutes.
7. Wash the wells as described in Step 4 above.
8. Place 100 µl of Substrate into each well, avoiding bubble formation.
9. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
10. Place 100 µl of Substrate into each well, avoiding bubble formation.
11. Read the absorbance of each well at 405 nm and zero against the reagent blank. A suitable reference wavelength (e.g., 600-630 nm) reading should be used. Read the plate within 60 minutes of adding Stop Solution.

Automated EIA Processor Users:
If using an Automated EIA Processor, refer to the Operator’s Manual for the test setup and procedures.

NOTE: Automated EIA Processor users must validate their equipment to demonstrate that the results obtained are equivalent to those obtained using manual assay.

Quality Control

1. Do not mix or interchange wells, controls, or calibrators from different lots.
2. Do not use reagents beyond their expiration date. Expiration dates are printed on the reagent labels.
3. Incubations above or below the recommended temperatures or times may give erroneous results.
4. The ELISA method is a very sensitive technique. Maintain consistent pipetting technique, incubation times, and temperature conditions throughout the test procedure. Cross contamination between reagents can invalidate the test.
5. Antigen coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
6. (Manual Procedure Only) The washing procedure is very important and requires special attention. (Please refer to the Procedure section)

NOTE: Improperly washed wells may give erroneous results.

Calculation

Determine the EU/ml (ELISA Units/ml) for each patient specimen or control using the following formula:

\[
\text{EU/ml Of sample} = \left( \frac{\text{EU/ml Of Calibrator}}{\text{Absorbance Of Calibrator}} \right) \times \text{Absorbance of sample}
\]

Reference Values

Antibodies to SSA occur in about 30 to 40% of patients with SLE and in about 60 to 70% of patients with Sjogren’s Syndrome. Up to 5% of the normal population may have antibodies to SSA antigen. SSA antibodies occurring alone have been associated with nephritis.

The expected values in the normal population were determined by assaying 100 normal donor sera collected in South Florida. Figures 1 and 3 show the distribution of SSA results in the normal population performed manually and on MAGO respectively. The distribution of EU/ml values for 65 clinically characterized sera along with the 100 normal donor sera is shown in Figures 2 and 4 performed manually and on MAGO respectively.
Figure 1 – Manual

anti-SSA Normals

Number of Sera

Figure 2 – Manual

anti-SSA Expected Values

Number of Sera
Figure 3 – MAGO

Figure 4 – MAGO

anti-SSA Expected Values
Interpretation of Results

**Single Point Calibration**

The anti-SSA Test Kit has been developed using a single point calibrator. Patient values which contain very high levels of antibody may produce absorbance values greater than the Calibrator absorbance. Patient sample results greater than the Calibrator value should be reported as “Greater than Calibrator value EU/ml”. If numerical results are required for such samples, dilute the sample using Sample Diluent and re-assay. Several dilutions (for example 1/10, 1/50 and 1/100) of the pre-diluted sample may be re-assayed simultaneously. Select the dilution that has an absorbance reading about 50% of the absorbance reading of the Calibrator; calculate the EU/ml for this dilution and multiply by the dilution factor to obtain estimated values.

**Test Validation Criteria**

1. The Positive Control must be within its assigned range.
2. The Negative Control must be < 16 EU/ml.
3. The absorbance of the reagent blank must be < 0.30.

*If any of these criteria are not met, the run is invalid and must be repeated.*

**Interpretation**

The following is a guide to interpretation of results. Each laboratory is encouraged to establish its own "normal" ranges based on populations encountered.

<table>
<thead>
<tr>
<th>anti-SSA Value</th>
<th>Index Value*</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 16 EU/ml</td>
<td>&lt; 0.8</td>
<td>Negative for antibodies to SSA.</td>
</tr>
<tr>
<td>16-20 EU/ml</td>
<td>0.8-1.0</td>
<td>Equivocal for antibodies to SSA. Sample should be retested. If retest results are equivocal, the sample should be reported as equivocal, tested by another method, or a new sample should be tested.**</td>
</tr>
<tr>
<td>&gt;20 EU/ml</td>
<td>&gt; 1.0</td>
<td>Positive for antibodies to SSA.</td>
</tr>
</tbody>
</table>

**Sensitivity**

Manual: 93%
MAGO: 93%

**Specificity**

**Specificty**

Manual: 99%
MAGO: 98%

**Crossreactivity**

Twenty-four sera positive for the six autoimmune specificities were tested in the anti-SSA Test Kit. The results are shown in Table 3. Since anti-SSB antibodies are nearly always accompanied by anti-SSA antibodies, samples 5-8 are positive in the anti-SSA test due to the additional presence of anti-SSA antibodies.
Table 3 Crossreactivity

<table>
<thead>
<tr>
<th>Sample</th>
<th>anti-SSA EU/ml</th>
<th>Interp</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>154.8</td>
<td>POS</td>
<td>SSA</td>
</tr>
<tr>
<td>2</td>
<td>204</td>
<td>POS</td>
<td>SSA</td>
</tr>
<tr>
<td>3</td>
<td>54.8</td>
<td>POS</td>
<td>SSA</td>
</tr>
<tr>
<td>4</td>
<td>203.6</td>
<td>POS</td>
<td>SSA</td>
</tr>
<tr>
<td>5</td>
<td>203.7</td>
<td>POS</td>
<td>SSB</td>
</tr>
<tr>
<td>6</td>
<td>204.7</td>
<td>POS</td>
<td>SSB</td>
</tr>
<tr>
<td>7</td>
<td>203.9</td>
<td>POS</td>
<td>SSB</td>
</tr>
<tr>
<td>8</td>
<td>203.7</td>
<td>POS</td>
<td>SSB</td>
</tr>
<tr>
<td>9</td>
<td>31.6*</td>
<td>POS</td>
<td>Sm</td>
</tr>
<tr>
<td>10</td>
<td>4.4</td>
<td>NEG</td>
<td>Sm</td>
</tr>
<tr>
<td>11</td>
<td>1.8</td>
<td>NEG</td>
<td>Sm</td>
</tr>
<tr>
<td>12</td>
<td>3.6</td>
<td>NEG</td>
<td>Sm</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>anti-SSA EU/ml</th>
<th>Interp</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>6</td>
<td>NEG</td>
<td>RNP</td>
</tr>
<tr>
<td>14</td>
<td>18.2**</td>
<td>EQ</td>
<td>RNP</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>NEG</td>
<td>RNP</td>
</tr>
<tr>
<td>16</td>
<td>6.8</td>
<td>NEG</td>
<td>RNP</td>
</tr>
<tr>
<td>17</td>
<td>2.5</td>
<td>NEG</td>
<td>Jo-1</td>
</tr>
<tr>
<td>18</td>
<td>5.1</td>
<td>NEG</td>
<td>Jo-1</td>
</tr>
<tr>
<td>19</td>
<td>4.3</td>
<td>NEG</td>
<td>Jo-1</td>
</tr>
<tr>
<td>20</td>
<td>2.6</td>
<td>NEG</td>
<td>Jo-1</td>
</tr>
<tr>
<td>21</td>
<td>4.1</td>
<td>NEG</td>
<td>Scl-70</td>
</tr>
<tr>
<td>22</td>
<td>6.4</td>
<td>NEG</td>
<td>Scl-70</td>
</tr>
<tr>
<td>23</td>
<td>2.1</td>
<td>NEG</td>
<td>Scl-70</td>
</tr>
<tr>
<td>24</td>
<td>6.5</td>
<td>NEG</td>
<td>Scl-70</td>
</tr>
</tbody>
</table>

**Linearity**

Figures 5 and 6 show typical examples of anti-SSA Test Kit linearity. The figures depict the results of the Calibrator tested by anti-SSA after a serial two-fold manual dilution in Sample Diluent. Separate dilutions were tested both manually and with MAGO. The results demonstrate a high degree of linearity for the anti-SSA Test Kit throughout the testing range.
Figure 5 - Manual

anti-SSA Calibrator Linearity

Absorbance

R² = 0.9892

Dilution

Figure 6 - MAGO

anti-SSA Calibrator Linearity

Absorbance

R² = 0.9795

Dilution
Reproducibility

The precision of the anti-SSA Test Kit was determined by testing six different sera and kit Calibrator and controls in two runs on three different days. The intra- and inter assay precision is shown in the table below.

<table>
<thead>
<tr>
<th>SERUM</th>
<th>Overall MEAN EU/ml</th>
<th>MANUAL INTRA-CV %</th>
<th>MANUAL INTER-CV%</th>
<th>MAGO INTRA-CV %</th>
<th>MAGO INTER-CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (NEG)</td>
<td>2.0</td>
<td>14.5</td>
<td>22.2</td>
<td>16.3</td>
<td>13.0</td>
</tr>
<tr>
<td>2 (NEG)</td>
<td>2.4</td>
<td>6.5</td>
<td>20.0</td>
<td>11.1</td>
<td>12.5</td>
</tr>
<tr>
<td>3 (POS)</td>
<td>46.7</td>
<td>4.1</td>
<td>6.3</td>
<td>3.9</td>
<td>5.2</td>
</tr>
<tr>
<td>4 (POS)</td>
<td>23.6</td>
<td>6.5</td>
<td>8.5</td>
<td>6.6</td>
<td>10.4</td>
</tr>
<tr>
<td>5 (POS)</td>
<td>65.6</td>
<td>5.0</td>
<td>8.2</td>
<td>2.3</td>
<td>5.2</td>
</tr>
<tr>
<td>6 (POS)</td>
<td>104.5</td>
<td>4.4</td>
<td>7.5</td>
<td>2.4</td>
<td>4.4</td>
</tr>
<tr>
<td>CAL</td>
<td>100.1</td>
<td>4.8</td>
<td>5.8</td>
<td>6.2</td>
<td>8.6</td>
</tr>
<tr>
<td>POS CTRL</td>
<td>42.6</td>
<td>3.1</td>
<td>5.0</td>
<td>5.0</td>
<td>7.4</td>
</tr>
<tr>
<td>NEG CTRL</td>
<td>1.5</td>
<td>9.1</td>
<td>21.4</td>
<td>24.9</td>
<td>25.0</td>
</tr>
</tbody>
</table>

Precautions

REAGENTS:
1. Handle samples, calibrators, controls and the materials that contact them as potential biohazards. Each donor unit in the Calibrator and controls has been found negative for Hepatitis B surface antigen and HIV-I antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1, Hepatitis B virus, or other infectious agents are absent, these materials should be handled at the Biosafety Level 2, as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual, “Biosafety in Microbiological and Biomedical Laboratories”, 1988.
2. Never pipette by mouth.
3. Avoid contact with open skin.
4. Certain of the test reagents contain 300 as a preservative. When disposing of reagents containing 300, flush drains with copious amounts of water to dilute the active components below active levels.
5. Reagents containing Sodium Azide:
   a) **CAUTION**: Some reagents in this kit contain Sodium Azide as preservative. Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to “Decontamination of Laboratory Sink Drains to Remove Azide Salts”, in the Manual Guide – Safety Management No. CDC-22, issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976. European Communities Hazardous Substance Risk and Safety Phrases (Council Directive 1999/45/EC)
   R28 – Very toxic if swallowed.
   R32 – Contact with acids liberates very toxic gas.
   R50/53 – Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
   S1/2 – Keep locked up and out of reach of children.
   S28 – After contact with skin, wash immediately with plenty of water and soap.
   S45 – In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
   S60 – This material and/or its container must be disposed of as hazardous waste.
   S61 – Avoid release to the environment. Refer to special instructions/Material Safety Data Sheet.
(b) Sodium Azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.

**Limitations**

1. The analysis of a single serum sample should not be used as the sole criterion for diagnosis of an autoimmune disease.
2. The results obtained with the anti-SSA Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
3. The test should be performed on serum. The use of whole blood or plasma has not been established.

**REFERENCES**