ANA Screen (Antinuclear Antibody) ELISA Kit

( Catalog # E4708-100; 96 assays; Storage at 4°C )

I. Introduction:
ANA Screen ELISA test system is an enzyme-linked immunosorbent assay (ELISA) for the detection of IgG class antibodies to ANA in human serum or plasma. Diluted patient serum is added to the ELISA plate coated with purified nuclear antigens. ANA IgG specific antibody binds to the antigen present in the samples. The enzyme conjugate is added to bind to the antibody-antigen complex. After addition of substrate the plate is incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

II. Applications:
- Detection of Antinuclear Antibody in human serum samples

III. Sample Type:
- Plasma
- Serum

IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>E4708-100</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with nuclear antigens</td>
<td>12 x 8</td>
<td>E4708-100-1</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>22 ml</td>
<td>E4708-100-2</td>
</tr>
<tr>
<td>Calibrator</td>
<td>1 ml</td>
<td>E4708-100-3</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1 ml</td>
<td>E4708-100-4</td>
</tr>
<tr>
<td>Negative Control</td>
<td>1 ml</td>
<td>E4708-100-5</td>
</tr>
<tr>
<td>Enzyme conjugate</td>
<td>12 ml</td>
<td>E4708-100-6</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>12 ml</td>
<td>E4708-100-7</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 ml</td>
<td>E4708-100-8</td>
</tr>
<tr>
<td>Wash buffer (20X)</td>
<td>25 ml</td>
<td>E4708-100-9</td>
</tr>
</tbody>
</table>

V. User Supplied Reagents and Equipment:
- Distilled or deionized water
- Microplate reader capable of measuring absorbance at 450 nm

VI. Storage Conditions and Reagent Preparation:
The entire kit may be stored at 4°C.

- **Wash buffer:** Prepare 1X Wash buffer by adding to 475 ml of distilled or deionized water to 25 ml (20X) buffer. Store at room temperature.
- **Sample preparation:** Collect blood specimens and separate the serum. Viscous forensic samples should always be diluted in phosphate buffered saline or distilled water prior to pipetting.

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.

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. Mix well.
3. Add 100 μl of diluted serum samples, calibrator, and controls into the appropriate wells. For blank, add 100 μl sample diluent in the well. Tap the plate to remove air bubbles and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 μl of 1X wash buffer. Blot on 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 1X wash buffer. Blot 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 a reference filter of 600-650 nm.

**Calculation:** Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value on every kit.

1. **Calculate the cut-off value:** Calibrator OD x Calibrator Factor (CF).
2. Calculate the Antibody Index of each determination by dividing the O.D. value of each sample by cut-off value.

**VIII. Related Products:**
- C-Peptide ELISA Kit (E4701)
- Creatinine (Human) ELISA Kit (E4368)
- C-Reactive Protein (CRP) (Human) ELISA Kit (E4289)
- Alpha-Fetoprotein (AFP) ELISA (K4242)

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