**INTENDED USE**

ENA screen (Extractable Nuclear Antigen) ELISA assay kit is used for the semi-quantitative determination of autoantibodies to nuclear and cytoplasmic antigens in human serum and plasma.

ENA screen (Extractable Nuclear Antigen) ELISA assay kit allows the simultaneous detection of autoantibodies to the extractable nuclear antigens SS-A(Ro), SS-B(La), Sm, RNP and Scl-70 as well as the cytoplasmic antigen Jo-1 in one sample.

ENA screen (Extractable Nuclear Antigen) ELISA assay kit offers a rapid and handsome opportunity for the determination of the whole autoantibody pattern in systemic autoimmune diseases on one test plate. The use of specified recombinant antigens in combination with selected highly purified ones guarantees a maximum of specificity for these parameters.

**PRINCIPLE OF THE TEST**

ENA screen (Extractable Nuclear Antigen) ELISA assay kit is an enzyme immunoassay for the semi-quantitative determination of IgG antibodies to nuclear and cytoplasmic antigens.

Antibodies of the calibrator and diluted samples react with nuclear and cytoplasmic antigens immobilized on the solid phase of microtiter plates. Recombinant SS-B, Sm, RNP (68 kDa, A, C), Scl-70, Jo-1 as well as highly purified Sm and SS-A guarantee the specific binding of autoimmune antibodies of the specimen under investigation. Following an incubation period of 60 min at room temperature (RT), unbound sample components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at RT, excessive conjugate is separated from the solid-phase immune complexes by the following wash step. HRP converts the colorless substrate solution of 3,3′,5,5′-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The cut-off is established by multiplying the OD of the calibrator with the corresponding factor.
**Specimen collection and storage**
Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires -20 °C. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20 °C.

**Preparation before use**
Allow samples to reach room temperature prior to assay. Take care to agitate samples gently in order to ensure homogeneity.

**TEST COMPONENTS FOR 96 DETERMINATIONS**

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Microtiter plate, 12 breakable strips per 8 wells (total 96 individual wells) coated with RNP (68kDa, A, C), SS-A, SS-B, Scl-70, Jo-1 and Sm</td>
</tr>
<tr>
<td>B</td>
<td>Concentrated wash buffer sufficient for 1000 ml solution</td>
</tr>
<tr>
<td>C</td>
<td>Sample diluent</td>
</tr>
<tr>
<td>D</td>
<td>Conjugate containing anti-human-IgG coupled with HRP</td>
</tr>
<tr>
<td>E</td>
<td>Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide</td>
</tr>
<tr>
<td>F</td>
<td>Stop solution 0.25 M sulfuric acid</td>
</tr>
<tr>
<td>Calibrator</td>
<td>(diluted serum) factor: see leaflet enclosed</td>
</tr>
<tr>
<td>N</td>
<td>Negative control (diluted serum)</td>
</tr>
</tbody>
</table>

**Materials required**
- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- trough for multi-channel pipette
- pipette tips
- glassware
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 or 690 nm
- distilled or de-ionized water

**Size and storage**
ENA screen (Extractable Nuclear Antigen) ELISA assay kit has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete ENA screen (Extractable Nuclear Antigen) ELISA assay kit on the box labels.

Upon receipt, all components of ENA screen (Extractable Nuclear Antigen) ELISA assay kit N have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

**Preparation before use**
Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash solution 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water. The wash solution prepared is stable at 2 - 8°C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

All other ENA screen (Extractable Nuclear Antigen) ELISA assay kit components are ready for use.

Avoid exposure of the TMB substrate solution to light!
**ASSAY PROCEDURE**

- Dilute sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl sample + 1 ml sample diluent (C).
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense
   - 100 µl calibrator (Ca)
   - 100 µl negative control (N)
   - 100 µl diluted samples into the respective wells.
3. Cover plate, incubate 60 min at room temperature (18…25°C).
4. Decant, then wash each well three times using 300 µl wash solution (made of B).
5. Add 100 µl of conjugate (D) solution to each well.
6. Cover plate, incubate 30 min at room temperature (18…25°C).
7. Decant, then wash each well three times using 300 µl wash solution (made of B).
8. Add 100 µl of substrate (E) to each well.
9. Incubate 15 min protected from light at room temperature (18…25°C).
10. Add 100 µl of stop solution (F) to each well and mix gently.
11. Read the OD at 450 nm versus 620 or 690 nm within 30 min after adding the stop solution.

**DATA PROCESSING**

Results are interpreted qualitatively by calculating a cut-off value (A) or semi-quantitatively by calculating the binding index (BI) for each sample (B) on the basis of the cut-off determined:

\[ \text{OD}_{\text{calibrator}} \times \text{factor} = \text{OD}_{\text{cut-off antigen}} \]

The factor for calculation is stated in the control certificate provided in the kit. The factor value may vary from lot to lot.

Example:
\[
\begin{align*}
\text{OD}_{\text{calibrator}} & = 0.982 \\
\text{factor} & = 0.4 \\
\text{OD}_{\text{cut-off}} & = 0.982 \times 0.4 = 0.393
\end{align*}
\]

(B) For the calculation of the binding index (ratio) the following formula should be applied:

\[ \text{BI} = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{cut-off}}} \]

Example:
\[
\begin{align*}
\text{OD}_{\text{cut-off}} & = 0.393 \\
\text{OD}_{\text{sample}} & = 1.756 \\
\text{BI} & = 1.756/0.393 = 4.5
\end{align*}
\]

This calculation can be performed by the integrated evaluation software of most microplate readers used, too.
REFERENCE VALUES

<table>
<thead>
<tr>
<th>ENA screen (Extractable Nuclear Antigen) ELISA</th>
<th>BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>≥ 1.0</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt; 1.0</td>
</tr>
</tbody>
</table>

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Test validity

The test run is valid if:

- the mean OD of the calibrator is ≥ 0.7
- the mean OD of the negative control ≤ 0.3

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

PERFORMANCE CHARACTERISTICS

Calibration

Due to the lack of international reference results are interpreted by calculating a BI (ratio).

Sensitivity

The analytical sensitivity of each readivity of the ENA screen (Extractable Nuclear Antigen) ELISA is around 0.2.

Precision

Intraassay variation (n=8)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean OD</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.589</td>
<td>0.03</td>
<td>5.1</td>
</tr>
<tr>
<td>2</td>
<td>1.149</td>
<td>0.05</td>
<td>4.3</td>
</tr>
<tr>
<td>3</td>
<td>1.945</td>
<td>0.06</td>
<td>3.1</td>
</tr>
</tbody>
</table>

Interassay variation (n=4x8)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean OD</th>
<th>SD</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.487</td>
<td>0.03</td>
<td>6.7</td>
</tr>
<tr>
<td>2</td>
<td>1.225</td>
<td>0.04</td>
<td>3.3</td>
</tr>
<tr>
<td>3</td>
<td>2.011</td>
<td>0.08</td>
<td>4.0</td>
</tr>
</tbody>
</table>
## INCUBATION SCHEME

### ENA screen (Extractable Nuclear Antigen) ELISA

<table>
<thead>
<tr>
<th>Step</th>
<th>Action</th>
<th>Volume(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bring all ready for use reagents to room temperature (18…25°C) before use.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Pipette, Calibrator (Ca)</td>
<td>calibrator: 100 µl; control: 100 µl; sera: 100 µl</td>
</tr>
<tr>
<td></td>
<td>Pipette, Negative Control (N)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pipette, prediluted 1 + 100 sera</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Incubate</td>
<td>60 minutes at room temperature</td>
</tr>
<tr>
<td>4</td>
<td>Wash</td>
<td>Decant, Dispense 3 x 300 µl (made of B)</td>
</tr>
<tr>
<td>5</td>
<td>Pipette conjugate (D)</td>
<td>100 µl; 100 µl; 100 µl</td>
</tr>
<tr>
<td>6</td>
<td>Incubate</td>
<td>30 minutes at room temperature</td>
</tr>
<tr>
<td>7</td>
<td>Wash</td>
<td>Decant, Dispense 3 x 300 µl (made of B)</td>
</tr>
<tr>
<td>8</td>
<td>Pipette substrate (E)</td>
<td>100 µl; 100 µl; 100 µl</td>
</tr>
<tr>
<td>9</td>
<td>Incubate protected from light</td>
<td>15 minutes at room temperature</td>
</tr>
<tr>
<td>10</td>
<td>Pipette stop solution (F)</td>
<td>100 µl; 100 µl; 100 µl</td>
</tr>
<tr>
<td>11</td>
<td>Measure 450 nm versus 620 (690) nm</td>
<td></td>
</tr>
</tbody>
</table>
SAFETY PRECAUTIONS

- This ENA screen (Extractable Nuclear Antigen) ELISA is for research use only.

- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.

- Do not use or mix reagents from different lots.

- Do not use reagents from other manufacturers.

- All reagents should be kept at 2 - 8 °C in the original shipping container until use.

- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservatives. They must not be swallowed or allowed to come into contact with skin or mucosa.

- Source materials derived from human body fluids or organs used in the preparation of ENA screen (Extractable Nuclear Antigen) ELISA were tested and found negative for HBsAg and for HIV as well as HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all samples as if potentially hazardous.

- Since the ENA screen (Extractable Nuclear Antigen) ELISA assay kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.