ANAscreen
(Antinuclear Antibody)
ELISA

Catalog Number:
ANS31-K01

Enzyme immunoassay for the determination of
IgG antibodies to nuclear and cytoplasmic antigens
in human serum and plasma

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For Research Use Only (RUO). Not for use in clinical, diagnostic or
erapeutic procedures.
v. 1.0

INTENDED USE

ANAscreen is used for the semi-quantitative
determination of autoantibodies to nuclear and
cytoplasmic antigens in human serum and plasma.

Systemic autoimmune diseases such as systemic lupus
erythematosus, scleroderma, rheumatoid arthritis,
Sjögren’s syndrome, dermatomyositis, mixed connective
tissue disease are characterized by the appearance of a
variety of autoantibodies directed against components
of the cell nucleus.

Although significance and pathological relevance of
some auto-antibodies are not completely revealed yet,
the detection of auto-antibodies is widely established
and plays an important role in the diagnosis of systemic
autoimmune diseases (1,2,3).

ANAscreen allows the detection of total autoantibodies
to nuclear and cytoplasmic antigens in one sample as a
summary parameter in the diagnosis of systemic
autoimmune disorders. Antigenic combination of
complete HeLa nuclei with recombinant proteins and
purified native antigens guarantees a maximum of
sensitivity and specificity for the ANA detection.

(1) Tan EM.: Antibodies to nuclear antigens (ANA) and
their immunobiology and medicine. Adv Immunol 1982
33:167-240
(2) von Mühlen CA, Tan EM: Autoantibodies in the
diagnostic of systemic rheumatic diseases. Semin
Arthritis Rheum 1995 24:323-358
(3) Smeenk RJT: Antinuclear antibodies: cause of disease
or caused by disease? Rheumatol 2000 39:581-584

PRINCIPLE OF THE TEST

The Eagle Biosciences ANAscreen ELISA Assay Kit is an
enzyme immunoassay for the semi-quantitative
determination of IgG antibodies to nuclear and
cytoplasmic antigens.

Antibodies of the controls and diluted patient samples
react with nuclear and cytoplasmic antigens immobilized
on the solid phase of microtiter plates. Use of complete
HeLa nuclei enriched with recombinant and native
antigens guarantees the specific binding of autoimmunity
antibodies of the specimen under investigation.
Following an incubation period of 60 min at room
temperature (RT, 18…25°C), 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. Patient ratios are calculated by dividing the
respective OD of the specimen with the calculated cut-off
OD.
**PATIENT SAMPLES**

**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.

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 patient samples gently in order to ensure homogeneity.

**Note:** Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1 ml sample diluent (C), prior to assay.

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires - 20 °C.

**TEST COMPONENTS FOR 96 DETERMINATIONS**

<table>
<thead>
<tr>
<th>Component</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A</strong></td>
<td>Microtiter plate, 12 breakable strips per 8 wells (total 96 individual wells) coated with HeLa nuclei and enriched with recombinant and native antigens</td>
</tr>
<tr>
<td><strong>Ag</strong></td>
<td>1 vacuum sealed with desiccant, 2 adhesive foils</td>
</tr>
<tr>
<td><strong>B</strong></td>
<td>Concentrated wash buffer sufficient for 1000 ml solution</td>
</tr>
<tr>
<td><strong>BUF</strong></td>
<td>100 ml capped white</td>
</tr>
<tr>
<td><strong>WASH</strong></td>
<td></td>
</tr>
<tr>
<td><strong>C</strong></td>
<td>Sample diluent</td>
</tr>
<tr>
<td><strong>DIL</strong></td>
<td>100 ml ready for use capped black</td>
</tr>
<tr>
<td><strong>D</strong></td>
<td>Conjugate containing anti-human-IgG coupled with HRP</td>
</tr>
<tr>
<td><strong>CONJ</strong></td>
<td>15 ml ready for use capped red</td>
</tr>
<tr>
<td><strong>E</strong></td>
<td>Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide</td>
</tr>
<tr>
<td><strong>SOLN</strong></td>
<td>15 ml ready for use capped blue</td>
</tr>
<tr>
<td><strong>TMB</strong></td>
<td></td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>Stop solution 0.25 M sulfuric acid</td>
</tr>
<tr>
<td><strong>H2SO4</strong></td>
<td>15 ml ready for use capped yellow</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>Positive control (diluted serum)</td>
</tr>
<tr>
<td><strong>CONTROL</strong></td>
<td>1 ml ready for use capped red</td>
</tr>
<tr>
<td><strong>CO</strong></td>
<td>Cut-off control (diluted serum)</td>
</tr>
<tr>
<td><strong>CONTROL</strong></td>
<td>1 ml ready for use capped white</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>Negative control (diluted serum)</td>
</tr>
<tr>
<td><strong>CONTROL</strong></td>
<td>1 ml ready for use capped green</td>
</tr>
</tbody>
</table>

**Materials required in addition**

- micropipettes
- multi-channel pipette or multi-pipette trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- distilled or de-ionized water
- glassware

**Size and storage**

ANAscreen has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the ANAscreen 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 buffer 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.

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

- Dilute patient 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 controls P, CO, N 100 µl diluted patient 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 or semi-quantitatively by calculating the binding index (BI) for each sample on the basis of the OD of the cut-off control:

\[
BI = \frac{OD_{sample}}{OD_{cut-off\ control}}
\]

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

\[
BI = \frac{OD_{sample}}{OD_{cut-off\ control}}
\]

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

### Example of Typical Assay Results

<table>
<thead>
<tr>
<th>well</th>
<th>OD (a)</th>
<th>OD (b)</th>
<th>OD (mean)</th>
<th>BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive control</td>
<td>1.558</td>
<td>1.510</td>
<td>1.534</td>
<td></td>
</tr>
<tr>
<td>Cut-off control</td>
<td>0.521</td>
<td>0.530</td>
<td>0.526</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>0.089</td>
<td>0.092</td>
<td>0.091</td>
<td></td>
</tr>
</tbody>
</table>

| Patient 1 | 1.604 | 1.599 | 1.602 | 3.1 - pos |
| Patient 2 | 1.261 | 1.287 | 1.274 | 2.4 - pos |
| Patient 3 | 0.170 | 0.157 | 0.164 | 0.3 - neg |

### Test validity

The test run is valid if:
- the mean OD of the positive control \( \geq 0.6 \)
- the mean OD of the cut-off control > mean OD of the negative control

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.

### REFERENCE VALUES

<table>
<thead>
<tr>
<th>ANAscreen</th>
<th>BI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>( \geq 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.
Limitations of Method

Healthy individuals should be tested negative by the ANAscreen. However, ANA positive apparently healthy persons do occur.

Due to the preparation of HeLa cell nuclei the antigen used in the assay may contain cytoplasmic components. A positive reaction of e.g. antibodies to mitochondria is not excluded.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

**PERFORMANCE CHARACTERISTICS**

**Calibration**

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

**Sensitivity**

The analytical sensitivity of the ANAscreen is 0.05 (BI).

**Specificity**

Frequency distribution of ANA in ANAscreen: 97 unselected human sera from healthy donors were tested. All sera were found negative. This corresponds to a diagnostic specificity of 100%.

**Precision**

The intra-assay coefficient of variation (CV) was determined by 20 fold measurements.

<table>
<thead>
<tr>
<th>Intra-Assay n = 20</th>
<th>mean BI</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.3</td>
<td>0.041</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>0.048</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>0.038</td>
<td>4.4</td>
</tr>
</tbody>
</table>

The inter-assay coefficient of variation (CV) was determined by 10fold measurements in five independent runs.

<table>
<thead>
<tr>
<th>Inter-Assay n = 5 x 10</th>
<th>mean BI</th>
<th>SD</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14.3</td>
<td>0.031</td>
<td>4.3</td>
</tr>
<tr>
<td></td>
<td>6.7</td>
<td>0.016</td>
<td>3.5</td>
</tr>
<tr>
<td></td>
<td>2.1</td>
<td>0.020</td>
<td>5.4</td>
</tr>
<tr>
<td>Step</td>
<td>Action</td>
<td>Reagents</td>
<td>Amount</td>
</tr>
<tr>
<td>------</td>
<td>--------</td>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td>1</td>
<td>Dilute patients sample</td>
<td>10 µl sample + 1 ml sample diluent (C)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Dispense positive control (P) cut-off control (CO) negative control (N) 1 + 100 diluted patient sera</td>
<td></td>
<td>100 µl</td>
</tr>
<tr>
<td>3</td>
<td>Incubate</td>
<td></td>
<td>60 min, room temperature (18...25°C)</td>
</tr>
<tr>
<td>4</td>
<td>Wash</td>
<td></td>
<td>Decant, 3 x 300 µl (made of B)</td>
</tr>
<tr>
<td>5</td>
<td>Dispense conjugate (D)</td>
<td></td>
<td>100 µl</td>
</tr>
<tr>
<td>6</td>
<td>Incubate</td>
<td></td>
<td>30 min, room temperature (18...25°C)</td>
</tr>
<tr>
<td>7</td>
<td>Wash</td>
<td></td>
<td>Decant, 3 x 300 µl (made of B)</td>
</tr>
<tr>
<td>8</td>
<td>Dispense substrate (E)</td>
<td></td>
<td>100 µl</td>
</tr>
<tr>
<td>9</td>
<td>Incubate in the dark</td>
<td></td>
<td>15 min, room temperature (18...25°C)</td>
</tr>
<tr>
<td>10</td>
<td>Dispense stop solution (F)</td>
<td></td>
<td>100 µl</td>
</tr>
<tr>
<td>11</td>
<td>Read at 450 against 620 (690) nm</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SAFETY PRECAUTIONS

- **This ANA screen ELISA Assay Kit is for research use only.** Follow the working instructions carefully. The kit should be performed by trained technical staff 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 Neolone M10 (< 1.0 % v/v) as preservative. 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 this kit 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 patient samples as if potentially hazardous.

- Since the 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

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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.