ELISA kits available from ADI (see details at the web site)

Catalog# ProdDescription
2940-10 Human C1q ELISA Kit, 96 tests
2950 Human Anti-C1q IgG ELISA Kit, 96 tests
2960 Human Circulating Immune complexes (CIC) ELISA Kit, 96 tests
2970 Monkey Circulating Immune complexes (CIC) ELISA Kit, 96 tests
3000 Human Rheumatoid Factors IgM (RF) ELISA Kit, 96 tests, Semi-Quantitative
3100 Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105 Human anti-dsDNA IgM ELISA Kit, 96 tests, Quantitative
3115 Human anti-ssDNA IgG ELISA Kit, 96 tests, Quantitative
3205 Human Anti-Nuclear Antibodies (ANA) ELISA Kit, 96 tests, Semi-Quantitative
3210-SSA Human anti-SS-A (60 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3215-SSA Human anti-SS-A (52 Kda/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3110 Human anti-dsDNA IgA ELISA Kit, 96 tests, Quantitative
3300-100-SMG Human Anti-Smith antigen (Sm) IgG ELISA kit, 96 tests, Quantitative
3300-110-SRG Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
3300-120-RNG Human Anti-RNP (RNP-70) IgG ELISA kit, 96 tests, Quantitative
3300-130-HNG Human Anti-histones IgG ELISA kit, 96 tests, Quantitative
3300-140-SCG Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA,
3300-150-JOG Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG ELISA kit,
3300-160-AFG Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG Human Anti-Cardioliopin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM Human Anti-Cardioliopin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA Human Anti-Cardioliopin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-B2M Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-B2A Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-205-APS Human Anti-Phospholipid Screen (anti-Phospholipid Serine, Phospholipid Inositol, Phosphatidic Acid and beta-2-Glycoprotein I) IgG/IgM ELISA kit, 96 tests, Quantitative
3300-210-PSS Human Anti-Phospholipid serine IgG/IgG ELISA kit, 96 tests, Quantitative
3300-215-PIS Human Anti-Phospholipid Inositol IgG/IgM ELISA kit, 96 tests, Quantitative
3300-220-PAS Human Anti-Phospholipid Acid IgG/IgM ELISA kit, 96 tests, Quantitative
3300-230-APG Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests, Quantitative
3300-235-APA Human Anti-Prothrombin IgA ELISA kit, 96 tests, Quantitative
3300-240-AVA Human Anti-Annexin V IgG ELISA kit, 96 tests, Quantitative
3300-250-ANG Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96 tests,
3300-255-PRG Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-260-LFG Human Anti-Lactofererin IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
3300-315-PRG Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell (H/K/ATPase) IgG ELISA kit, 96 tests, Quantitative
5120 Mouse anti-dsDNA IgG-specific ELISA Kit, 96 tests, Quantitative
5130 Mouse anti-dsDNA IgM-specific ELISA Kit, 96 tests, Quantitative
5210 Mouse Anti-Nuclear Antibigens (ANA/ENA) IgG's (total A+G+M ) ELISA Kit, 96 tests,
5320 Mouse Anti-ssDNA IgG-specific ELISA Kit, 96 tests, Quantitative
5330 Mouse Anti-ssDNA IgM-specific ELISA Kit, 96 tests, Quantitative
5405 Mouse Anti-Smith IgG's (total A+G+M) ELISA kit, 96 tests, Quantitative
5415 Mouse Anti-RNP IgG ELISA Kit, 96 tests, Quantitative
5420 Mouse Anti-rRNP IgM ELISA Kit, 96 tests, Quantitative
5520 Rat Anti-Cardioliopin IgG's (A+G+M) ELISA kit, 96 Tests, Quantitative
5610 Mouse Anti-Histones IgG's (total A+G+M) ELISA kit, 96 tests, Quantitative
5710 Mouse Anti-SSA/Ro IgG's (total A+G+M) ELISA kit, 96 tests, Quantitative
5810 Mouse Anti-SSB IgG's (total A+G+M) ELISA kit, 96 tests, Quantitative
5900 Mouse Circulating Immune Complexes (CIC) IgG's (total A+G+M) ELISA kit, 96 Tests,
5950 Rat Circulating Immune Complexes (CIC) IgG's (total A+G+M) ELISA kit, 96 Tests,
Rheumatoid autoimmune diseases are often associated with the occurrence of autoantibodies against several nuclear or cytoplasmatic antigens. These so-called anti nuclear antigens (ANA) can be divided into three groups:

1. true anti nuclear antigens (ANA): dsDNA, ssDNA, histones, nucleic RNA and DNP
2. extractable nucleas antigens: Sm (Smith), n-RNP, Scl 70 and PM-1
3. cytoplasmatic antigens: SS-A (Ro)*, SS-B (La)* and Jo-1 SS-A (Ro) and SS-B (La) are co-localized in cytoplasm and nucleus

Inflammatory connective tissue diseases are characterized by idiopathic genesis along with disturbances in terms of cellular and humoral immunity, systemic organ failure and a chronic course of disease. Additionally, connective tissue diseases exhibit overlapping symptomatic features that render an accurate diagnosis difficult. Considering the diversity of mixed connective tissue diseases, such disorders exhibit a common serological characteristic; the presence of anti-nuclear antibodies. These antibodies are directed against parts of the cell nucleus and the cytoplasm, and many rheumatic diseases are characterized by the presence of one or more of these ANAs. Antibodies to double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), histone, nuclear ribonucleoprotein (RNP) and Smith antigen (Sm) are associated with SLE, while antibodies to Sjogren’s Syndromes A (SSA/Ro) and B (SSB/La) can occur in both SLE and Sjogren’s Syndrome (SS). Antibodies to Jo-1 may be observed in polymyositis and dermatomyositis, while antibodies to scleroderma-associated antigen (Scl-70) and centromere can occur in patients with progressive systemic sclerosis (PSS). Anti-histone antibodies are associated with SLE and drug-induced lupus, while anti-RNP antibodies are linked with mixed connective tissue disease (MCTD) and with SLE. Antibodies directed against centromere are associated with CREST syndrome. Although IFA technology was traditionally used to detect autoantibodies in conjunction with HEp2 cells, it is now widely acknowledged that ELISA technology offers an excellent alternative.

Anti-Nuclear Antibodies (ANA) are autoantibodies which binds to cellular nuclear antigens including dsDNA, ss-DNA, histones, ribonucleoproteins (RNP) and the SS-A, SS-B, and Sm antigens. ANA ELISA, a sandwich ELISA, provides a rapid semi-quantitative measurement of ANA in serum to further investigate the presence of specific autoantibodies. Positive ANA may be found in apparently healthy people. It is therefore imperative that the results be interpreted in light of the patients clinical picture by a medical authority. SLE patients undergoing steroid therapy may have negative test results. Many common prescribed drugs may be induce ANA. The ANA screen test system will not identify the specific type of ANA present in a positive sample. Positive specimens should be tested for individual autoantibodies using the ANA autoantibody profile-6 or the ANA ENA profile-6 test systems.

**PERFORMANCE CHARAVEREISTICS**

**Parallelism**

Three dilutions of three patient samples were assayed using two kit batches. The following table shows the mean values and the dilution-corrected recovery.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Index Value</th>
<th>Dilution corrected recovery [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1/100</td>
<td>4.8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1/200</td>
<td>2.2</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>1/400</td>
<td>1.3</td>
<td>108</td>
</tr>
<tr>
<td>2</td>
<td>1/100</td>
<td>2.8</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1/200</td>
<td>1.5</td>
<td>107</td>
</tr>
<tr>
<td></td>
<td>1/400</td>
<td>0.8</td>
<td>114</td>
</tr>
<tr>
<td>3</td>
<td>1/100</td>
<td>3.5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>1/200</td>
<td>1.7</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>1/400</td>
<td>0.8</td>
<td>91</td>
</tr>
</tbody>
</table>

**Precision (Reproducibility)**

Statistics for coefficients of variation (CV) were calculated for each of four samples results of 32 determinations in a single run for Intra-Assay precision. Run-to-run prec calculated from the results of 3 different runs with 24 determinations of each sample.

<table>
<thead>
<tr>
<th>Intra-Assay</th>
<th>Inter-Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample No</td>
<td>Mean (Index Value)</td>
</tr>
<tr>
<td>1</td>
<td>1.815.9</td>
</tr>
<tr>
<td>2</td>
<td>2.4</td>
</tr>
<tr>
<td>3</td>
<td>2.8</td>
</tr>
<tr>
<td>4</td>
<td>3.1</td>
</tr>
</tbody>
</table>

**Performance Comparison**

ADA ANA ELISA kit was compared with a commercially available ELISA assay (94 samples). The assay show relative sensitivity of 93%, specificity (96%) and agreement (95%).

INTERPRETAION AND CALCULATION OF RESULTS

For detailed semi-quantitative results, each patient-OD value can be expressed as an "Index Value". The Index Value is calculated by dividing the sample-OD by the cut-off-OD:

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

The calculation of Index Values is not influenced by variations of the sample-OD and/or cut-off-OD. Index Values are recommended for long term validations (i.e. internal quality control samples).

Interpretation of results

1. Evaluation of the ANA Detect ELISA test is easily carried out by direct comparison of the optical density of each patient sample with the optical density of the cut-off control (B). Patient samples exhibiting optical densities higher than the optical density of the cut-off control are considered to be positive.

   - **Negative:** OD patient < OD Cut-off
   - **Elevated:** OD patient > OD Cut-off

2. Index Values are interpreted as follows: ANA Detect ELISA:

   - **Negative:** (Index-value) < 1.0
   - **Borderline:** 1.0 - 1.2
   - **Positive:** 1.2

Example:

The table shows typical results for an ANA Detect ELISA assay. These data are intended for illustration only and should not be used to calculate results from a laboratory assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample OD</th>
<th>OD Cut-Off</th>
<th>Index Value</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.107</td>
<td>0.435</td>
<td>0.25</td>
<td>negative</td>
</tr>
<tr>
<td>2</td>
<td>0.435</td>
<td>0.435</td>
<td>1.00</td>
<td>borderline</td>
</tr>
<tr>
<td>3</td>
<td>1.294</td>
<td>0.435</td>
<td>2.97</td>
<td>positive</td>
</tr>
<tr>
<td>4</td>
<td>2.496</td>
<td>0.435</td>
<td>5.74</td>
<td>positive</td>
</tr>
</tbody>
</table>

Expected Values

The approximate incidence of positive ANA is 5% in the general normal population, 40% in normal old age and 25% in healthy relatives of SLE patients. ANA positivity has been reported in:

- **SLE** (systemic Lupus erythematosus) >95%
- **SS** (Sjögren’s syndrome) 50-65%
- **PSS** (progressive systemic sclerosis) 40-60%
- **RA** (rheumatoid arthritis) 12-24%
- **Juvenile RA** (juvenile rheumatoid arthritis) 20%

PRINCIPLE OF THE TEST

Anti-Nuclear Antibodies (ANA) ELISA kit is based on binding of ANA from serum samples to extracted nuclear antigen immobilized on microtiter wells. After a washing step, goat anti-human IgG-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of ANA present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of ANA in samples is calculated as ANA index (AI) which is defined as the ratio of net absorbance of the test sample and net absorbance of the negative or endpoint-cutoff control.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plate Reader.

PRECAUTIONS

The ADI’s. ANA ELISA test is intended for in vitro research use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The Endpoint Cutoff and Positive controls have been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable MSDS, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates).

All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum. If sera can not be immediately assayed, these could be stored at -20°C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

Preparation of the reagent:

Dilute wash buffer (1:50) with distilled water (20 ml stock in total of 1-liter). store at 40°C.

Sample buffer (1:5) with distilled water (20 ml stock in total of 100-ml). store at 40°C for 30-day or until the expiration date printed on the label.
REAGENT PREPARATION FOR THE ASSAY

1. Dilute wash buffer 1:50 (20 ml stock in 980 ml water) and store at 4°C.
2. Dilute sample diluent 1:5 (20 ml stock in 80 ml water) and store at 4°C.
3. Dilute all samples to be tested 1:100 with sample diluent (10 ul sample in 990 ul of diluent or 5 ul sample in 495 ul diluent).
4. Bring all reagents and samples to room temperature (25-30oC)

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate storage conditions.

Do not contaminate the bottles. Withdraw solutions in a separate clean tube or dispensing trays. Any unused solution should be discarded and not returned to the bottle. Do not use HRP substrate solution if this solution is blue. Do not expose these solutions to strong light.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE).

1. Label, and secure the microtiter well strips to be used on the plate.
   Dilute samples (1:100) in 1x sample diluent. Controls provided in the kit are already pre-diluted.

2. Pipet 100 ul of sample diluent (for use as blanks), pre-diluted negative, positive controls, and diluted serum samples into appropriate wells in duplicate. Mix gently for 5-10 seconds, cover the plate and incubate for 30 minutes at room temp (24-28oC).

3. Aspirate and wash the wells 3 times with 300 ul of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. Failure to wash the wells properly will lead to high blank values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

4. Add 100 ul of enzyme conjugate into each well. Mix gently for 5-10 seconds. Cover the plate and incubate for 15 minutes at room temp.

5. Aspirate and wash the wells 4 times as above.

6. Dispense 100 ul TMB substrate per well. Mix gently for 5 seconds. Cover the plate and incubate at room temp in the dark. for 15 minutes. Blue color develops in positive wells.

7. Stop the reaction by adding 100 ul of stopping solution to all wells at the same timed intervals. Mix gently for 5-10 seconds. Blue color turns yellow. Measure the absorbance at 450 nm using an ELISA reader.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence.

QUALITY CONTROL

This test is only valid if the optical density at 450 nm for negative control (NC), cut-off control (CC) and positive control (PC) complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated. The assays is calibrated against the internationally recognised reference sera from CDC, Atlanta, USA and furthermore against the WHO reference preparation for human anti-dsDNA Wo/80.

Each time the assay is run, the cutoff control must be run in triplicate. A positive and negative control must also be included in each assay.

Calculate the mean of the three cutoff controls. If any of the three values differs by more than 15% from the mean more than 15%, discard the value and calculate the mean of the remaining two values.

The mean OD value for the cut-off and the OD values for positive and negative controls should fall within the following ranges:

<table>
<thead>
<tr>
<th></th>
<th>A450 (OD range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>&lt;0.200</td>
</tr>
<tr>
<td>Cutoff control</td>
<td>&gt;Negative control</td>
</tr>
<tr>
<td>Positive control</td>
<td>≥0.500</td>
</tr>
</tbody>
</table>

(a) the OD values for the negative control divided by the mean OD of the cutoff control should be ≤0.900
(b) the OD value for the positive control divided by the mean value of the cutoff control should be ≥1.50
(c) IF the above conditions are not met, the test should be considered invalid and should be repeated.

Additional controls may tested according to guidelines or requirement of local, state and/or federal regulations or accredited organizations.