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-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM	Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA	Human Anti-Cardiolipin 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-Phosphatidyl Serine, Phosphatidyl Inositol, Phosphatidic Acid and beta-2-Glycoprotein I) IgG/IgM ELISA kit, 96 tests, Quantitative
3300-210-PSS	Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests, Quantitative
3300-215-PIS	Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests, Quantitative
3300-220-PAS	Human Anti-Phosphotidic 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-Lactoferrin 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 Antigens (ANA/ENA) Ig'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-Sm Ig's (total (A+G+M) ELISA Kit, 96 tests, Quantitative
5415	Mouse Anti-nRNP IgG ELISA Kit, 96 tests, Quantitative
5420	Mouse Anti-nRNP IgM ELISA Kit, 96 tests, Quantitative
5520	Rat Anti-Cardiolipin Ig's (A+G+M) ELISA kit, 96 Tests, Quantitative
5610	Mouse Anti-Histones Ig's (total (A+G+M) ELISA Kit, 96 tests, Quantitative
5710	Mouse Anti-SSA/Ro Ig's (total (A+G+M) ELISA Kit, 96 tests, Quantitative
5810	Mouse Anti-SSB Ig's (total (A+G+M) ELISA Kit, 96 tests, Quantitative
5900	Mouse Circulating Immune Complexes (CIC) Ig's (total (A+G+M) ELISA kit, 96 Tests,
5950	Rat Circulating Immune Complexes (CIC) Ig's (total (A+G+M) ELISA kit, 96 Tests,

Instruction Manual No. M-3205

Anti-Nuclear Antibody (ANA)

ELISA KIT Cat. No. 3205, 96 Tests

For Semi-Quantitative Determination of ANA In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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Phone (210) 561-9515 • Fax (210) 561-9544
Toll Free (800) 786-5777

Email: service@4adi.com Web Site: www.4adi.com

Anti Nuclear Antibodies (ANA) ELISA KIT #3205

For Semi-Quant. Determination of ANA In Human Serum: Kit Contents (96 tests):

Components	Cat. No.
Nuclear antigen coated microwell strips (96 wells)	3 2 0 6
ANA **Negative Control A**, ready-to-use, 1.5 ml	3 2 0 7 N
ANA **Cut-off Control B**, , ready-to-use, 1.5 ml	3 2 0 7 C
ANA **Positive Control C**, ready-to-use, 1.5 ml	3 2 0 7 P
Sample Diluent (5X), 20 ml (yellow color)	3 2 0 8
Wash buffer (50X), 20 ml	3205-WB
Anti-hIgG **HRP Conjugate**, 15 ml (light red)	3 2 0 5 - E C
HRP **Substrate Solution** (TMB) , 15 ml	3 2 0 5 - T M
Stop Solution, 15 ml	3 2 0 5 - S S
Complete Instruction Manual	M - 3 2 0 5

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, nucleolic RNA and DNP
2. extractable nuclears 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 Syndrome A (SSA/Ro) and Sjogren´s Syndrome 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 ds-DNA, 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 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.

Sample	Dilution	Index Value	Dilution corrected recovery [%]
1	1/100 1/200 1/400	4.8 2.2 1.3	100 92 108
2	1/100 1/200 1/400	2.8 1.5 0.8	100 107 114
3	1/100 1/200 1/400	3.5 1.7 0.8	100 97 91

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 preci calculated from the results of 3 different runs with 24 determinations of each sample:

Intra-Assay		
Sample No	Mean (Index Value)	CV [%]
1	1.815.9	6.9
2	2.4	9.1
3	2.8	10.4
4	3.1	7.4

Inter-Assay		
Sample No	Mean (Index Value)	CV [%]
1	1.6	13.7
2	3.7	10.4
3	4.1	11.2

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%).

References: Reichlin, M (1981) Clin. Exp. Immunol. 44, 1-10; Veneables PJW et al (1980) Clin. Exp. Immunol. (1980) 39, 16; Harmon CE et al (1985) Med. Clin. N. Am. 69, 547; Rothschild BM et al (1983) Arth. Rheum. 26, 45-51

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:

$$\text{Index Value} = \frac{OD_{sample}}{OD_{cut\text{-}off}}$$

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:

	(Index-value)
Negative:	< 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.

Sample.	Sample OD	OD Cut-Off	Index Value	Interpretation
1	0.107	0.435	0.25	negative
2	0.435	0.435	1.00	borderline
3	1.294	0.435	2.97	positive
4	2.496	0.435	5.74	positive

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^{o}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 4oC.
Sample buffer (1:5) with distilled water (20 ml stock in total of 100-ml). store at 4oC 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 4oC.
2. Dilute **sample diluent 1:5** (20 ml stock in 80 ml water) and store at 4oC.
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^{o}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 **ready-to-use**.

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:

	A450 (OD range))
Negative Control	<0.200
Cutoff control	>Negative control
Positive control	≥0.500

(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.