RELISA® ANA SCREENING TEST SYSTEM

For in vitro Diagnostic Use
For Professional Use
Catalog numbers: 7096-11 (96 wells) and 7696-11 (576 wells)

INTENDED USE: This is an enzyme immunoassay test system for the detection of antinuclear antibodies in human serum. This test system is to be used as an aid in the detection of antibodies associated with systemic rheumatic disease.

Summary and Explanation of the Test

Antinuclear antibody (ANA) is a general term used to describe autoantibodies against various cell nuclear proteins. Early studies of these autoantibodies, using immunofluorescent techniques, revealed a select few nuclear protein specificities (1). Because of the high correlation of positive ANA with systemic lupus erythematosus (SLE), a negative ANA essentially ruled out the disease (2).

Although antibodies specific to DNA continue to show a high disease correlation with SLE (3), a number of nuclear (4) and cytoplasmic (5-7) macromolecules have also been detected and associated with other connective tissue diseases (8-10). Some of these antibodies appear to have diagnostic and/or prognostic significance in progressive systemic sclerosis (11-12), mixed connective tissue disease (13-15), Sjögren's syndrome (16-17), polymyositis (18), and/or rheumatoid arthritis (19). Because of these disease associations, ANA testing is now recognized as a general screening tool for connective tissue disease (20).

The most common method used to test for ANA is the indirect fluorescent antibody (IFA) method using cultured cells. The sensitivity of these assays varies with the type of substrate used, fixative procedure and types of ANA present in the sera. The IFA test using HEp-2 or HEp-2000® cells is considered to be a sensitive test for the detection of ANA, however it is labor-intensive and subject to errors from variability in fluorescent microscopes and from human interpretation.

Enzyme immunoassays (EIA) are an alternative to the IFA method. EIA test systems can efficiently screen large numbers of samples. Human errors are reduced, and results are provided in an objective form. However, EIA methods have been reported to show both false positive and false negative results when compared to IFA methods (21).

Principle of the Test

This test is a qualitative indirect EIA. Stabilized antigens (dsDNA, histones, SSA/Ro, SSB/La, Sm, Sm/RNP, Scl-70, centromere, Jo-1, PCNA, Ribosomal P, M2 and other antigens extracted from the HEp-2 nucleus and cytoplasm) have been coated onto the surface of the microwells to serve as an antigenic substrate in this system. Dilutions of the patient samples are placed in the microwells and incubated, allowing specific antibodies in the sample to react with the antigen on the solid phase. After washing to remove unbound antibody and other serum proteins, the wells are incubated with goat anti-human antibodies that are labeled with horseradish peroxidase. The horseradish peroxidase-conjugated antibody preparation that is included in the test system is specific for human IgG heavy and light chains.
After incubation with horseradish peroxidase conjugate, a stable three-part complex is formed if results are positive. This complex consists of horseradish peroxidase-conjugated anti-human antibody bound to human antinuclear antibody, which is bound to the antigen stabilized on the plastic surface.

After another washing step, this complex is detected by adding a solution of tetramethylbenzidine (TMB) and H_2O_2 as a chromogenic substrate. The degree of color development in each well is proportional to the concentration of antinuclear antibodies in each serum sample. Each microwell is read in a spectrophotometer at 450 nm.

System Components - materials provided

Storage: All components should be stored under refrigeration between 2-10°C. Do not freeze.

Stability: All components remain stable at least 12 months from date of manufacture. Do not use any component beyond its expiration date.

REACTIVE REAGENTS

RELISA® Antigen Coated Microwell Strips PLATE: Catalog No. 7008-11. A microwell frame containing twelve eight well strips coated with cellular antigens (dsDNA, histones, SSA/Ro, SSB/La, Sm, Sm/RNP, Scl-70, centromere, Jo-1, PCNA, Ribosomal P, M2 and other antigens extracted from the HEp-2 nucleus and cytoplasm). These strips are color-coded plum. If fewer than eight wells are needed for testing, the wells can be separated by snapping them apart. The unused strips can be returned to the foil pouch with the desiccant pack, sealed with the zipper seal, and refrigerated for up to 45 days.

RELISA® Sample Diluent SOLN|DIL: Catalog No. 7100 (100 ml). Proprietary buffered sample diluent used to dilute patient samples.

RELISA® Enzyme Antibody Reagent - Human IgG heavy and light chain specific CONJ|HRP: Catalog No. 7009-11 (14 ml). Anti-human IgG (H&L) conjugated to horseradish peroxidase (HRP). Reagent is ready to use.

RELISA® Substrate Solution SOLN|SUB  : Catalog No. 7035 (14 ml). HRP-specific enzyme substrate solution, containing stabilized 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2). Reagent is ready to use. **CAUTION:** Flammable. This reagent contans less than 25% of methanol and acetone. Keep out of the reach of children. In case of contact with eyes, flush immediately and thoroughly with water and consult a physician.

RELISA® Stopping Reagent SOLN|STOP  : Catalog No. 7033 (14 ml). Proprietary stopping reagent for Immuno Concepts EIA test systems. Reagent is ready to use. **DANGER:** Corrosive. This reagent contains hydrochloric acid and should be handled with care. Keep out of the reach of children. In case of contact with eyes, flush immediately and thoroughly with water and consult a physician. Never add water to this reagent.

RELISA® ANA Calibrator Serum CAL: Catalog No. 7026-11 (2 ml). Human serum that contains antinuclear antibodies. The assay value for this serum is stated on the vial label. This serum is at working dilution and is ready to use.

RELISA® ANA Positive Control CONTROL| + : Catalog No. 7021-11 (2 ml). Human positive control serum that contains antinuclear antibodies. This serum is at working dilution and is ready to use.

RELISA® ANA Negative Control CONTROL| - : Catalog No. 7031 (2 ml). Human negative control serum that does not contain antinuclear antibodies. This serum is at working dilution and is ready to use.

RELISA® Optional ANA Undiluted Positive Control OPT+: Catalog No. 7022-11 (0.25 ml). Human positive control serum that contains antinuclear antibodies. Treat this positive control as an undiluted serum.

NON-REACTIVE COMPONENTS

Holder for microwells

Wash Buffer Solution:

PBS Buffer PWDR|PBS: Catalog No. 1011. Phosphate-buffered saline powder (0.01 M, pH 7.4 ± 0.2). Each pouch contains sufficient buffer powder to make one liter. (Two pouches of buffer powder are supplied for each 96-microwell plate in complete test kits).

Wash Buffer Concentrate SOLN|WASH: Catalog No. 1031 (10 ml). 5% Tween 20 solution to be used in the wash buffer. (Two vials of buffer concentrate are supplied for each 96-microwell plate in complete test kits).

Preparation: Dissolve one pouch of buffer powder in one liter of deionized or distilled water. Add the entire contents of one bottle of Wash Buffer Concentrate to the dissolved PBS. Mix well and store between 2-25°C for up to 4 weeks or until signs of contamination or other visible changes occur. Wash buffer solution must be at room temperature (18-25°C) before use.

Additional Materials Required - but not provided

Volumetric precision pipettors to deliver 25-1000 µl volumes
Squeeze bottle for delivering wash buffer solution to microwells, or an automated or semi-automated wash system for microwells
One-liter container for PBS wash buffer solution
Deionized or distilled water
Plate reading spectrophotometer capable of reading absorbance at 450 nm
Test tubes to prepare serum dilutions
Bibulous paper or paper towels
Multichannel pipettor capable of delivering to 8 wells
Disposable gloves
Lab timer

Precautions

1. All human source materials used for this product have been tested and found to be negative (not repeatedly reactive) for antibodies to Human Immunodeficiency Virus-1 (HIV-1), Human Immunodeficiency Virus-2 (HIV-2), hepatitis C virus (HCV), and for hepatitis B surface antigen (HBsAg) by FDA approved methods. However, no test method can offer complete assurance that HIV-1, HIV-2, hepatitis C, hepatitis B, or other infectious agents are absent. Thus, all kit materials should be handled in the same manner as potentially infectious materials.
2. All patient samples should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual: *Biosafety in Microbiological and Biomedical Laboratories, 1999 Edition.*
3. Dilution of the components or substitution of components other than those provided in this system may yield inconsistent results.
4. Sodium azide (0.09%) is used as a preservative. Sodium azide may react with lead or copper plumbing and form explosive metal azide salts. When disposing of reagents, flush with ample volumes of tap water to prevent potential residues in plumbing. Sodium azide is a poison and may be toxic if ingested.
5. This kit is for *in vitro* diagnostic use.
6. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.
7. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
8. Avoid splashing or generation of aerosols at all times.
9. Incubation times and temperatures other than those specified may give erroneous results.
10. Cross contamination of reagents or samples may give false results. Samples must remain confined to microwells during testing.
11. Reusable glassware must be washed and thoroughly rinsed free of detergents prior to use. All glassware must be clean and dry before use.
12. Bring all reagents, microwells, and specimens to room temperature (18-25°C) prior to use.
13. Wear disposable gloves when handling specimens and reagents, and wash hands thoroughly afterwards.
14. Microbial contamination of reagents or samples may give false results.
15. The stopping reagent is corrosive, and may cause burns. This reagent contains hydrochloric acid and should be handled with care. Keep out of the reach of children. In case of contact with eyes, flush immediately and thoroughly with water and consult a physician. Never add water to this reagent.

Specimen Collection

Collection: Serum is the preferred specimen. Approximately 5 ml of whole blood should be collected aseptically by venipuncture using a sterile vacuum collection tube or other suitable collection system. Allow blood to clot at room temperature (18-25°C). Serum should be separated from the clot by centrifugation as soon as possible to minimize hemolysis.

Interfering Substances: Sera exhibiting a high degree of hemolysis, icterus, lipemia, or microbial growth should not be used because these conditions may cause aberrant results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

Storage: Sera may be stored at 2-10°C up to one week. If testing is further delayed, sera should be stored frozen at –20°C or lower. Serum should not be stored in a self-defrosting refrigerator or freezer.

CAUTION: Repeated freeze/thawing of patient samples may yield false positive or false negative results.

General Procedural Notes

1. It is extremely important to have all kit components and serum samples at room temperature (18-25°C) before use. A full liter of wash buffer may require several hours to warm to 20°C after removal from the refrigerator. Incubation temperatures above or below the stated range may cause inaccurate results. Return unused samples and reagents to refrigerated storage after use.
2. Mix reagents well before use by gentle inversion. Do not vortex or shake reagents. Avoid foaming.
3. When preparing sample dilutions, pipette tips should be wiped prior to dispensing serum into specimen diluent. Excess sample adhering to the outside of the pipette tip will affect results.
4. The use of a multichannel pipettor is recommended because it provides more uniform reagent dispensing, incubation times, and reaction times.
5. **Adequate washing of wells is extremely important.** Inadequately washed wells will exhibit high background values, and may show false positive values. For manual washing, aspirate the contents of the wells, then fill each well with wash buffer solution. Avoid cross-contamination of the wells, particularly in the first wash after aspiration. Drain all of the wash buffer from the wells by inverting, then shaking residual wash buffer from the wells with a sharp “snapping” motion of the wrist. Repeat the filling and draining steps for a total of 3 to 5 washes. The wells should then be rapped vigorously on a paper towel or other absorbent material to remove all traces of residual wash buffer. The use of an automated microwell washing system will assure consistent washing of the wells, and is recommended.
   NOTE: Due to the various types of wash techniques and automated systems, the number of washes may be adjusted to obtain optimal results. Each laboratory should determine the most efficient number of washes for its washing system.
6. Inadequate removal of residual wash buffer can cause inconsistent color development. Microwell strips should be blotted on absorbent paper or towels to minimize residual wash buffer.
7. Timing of all steps is critical. All serum samples should be diluted before beginning the procedure, and they must be dispensed into the microwells in as short a period of time as possible (not more than five minutes). Batch sizes should be set so that specimen handling can be accomplished comfortably within this time period. The use of a multichannel pipettor facilitates the handling of samples and reagents, and is recommended.
8. With the exception of the last incubation (substrate solution), the start of each incubation period begins with completion of sample or reagent dispensing. The substrate solution incubation must be exactly 30 minutes for each well. All samples and reagents should be dispensed in the same sequence and at a constant rate.

Interpretation of Results

CALCULATIONS

1. Subtract the absorbance value for the reagent blank well from the absorbance values obtained in calibrator, control, and patient sample wells. Calculate the mean absorbance values for duplicate wells.
2. Divide the specific antibody concentration of the calibrator serum (stated on the label) by the mean absorbance value of the calibrator wells to obtain the Conversion Factor.
3. Multiply the absorbance values of each of the samples by the Conversion Factor to obtain the specific antibody concentration in units.

4. The simplified form of these calculations can be expressed as:

$$\frac{Uc}{\lambda c} \times \lambda s = Us$$

Uc = Calibrator Value (Units)
λc = Absorbance of Calibrator*
λs = Absorbance of Sample*
Us = Unit Value of Sample

*If calibrators and samples are run in duplicate, use the average absorbance of the duplicate wells.

QUALITY CONTROL

1. The mean absorbance value of the calibrator wells must be at least 0.400. Absorbance values less than 0.400 indicate inadequate color development, and an invalid run. Inadequate color development is usually due to use of cold reagents or incorrect timing of one or more steps of the assay. Allow reagents to warm to room temperature (18-25°C), and repeat the run with particular attention paid to the timing of all steps.
2. The blank control well should have an absorbance value of less than 0.150. Blank absorbance values greater than 0.150 indicate inadequate washing, or contamination of reagents.
3. Samples with specific antibody values greater than the upper limit of the calibrator should be reported as positive with a unit value “greater than or equal to” the unit value stated on the label of the calibrator serum.
4. The Conversion Factor must be calculated for each run. Using a Conversion Factor from another run will invalidate the results.
5. Each laboratory should establish and maintain its own reference (normal) range values, based on the patient population and other local factors.
6. The positive control serum is a human serum that contains antinuclear antibodies. This is a qualitative control, which should give a value of greater than 15 ANA units.
7. The negative control serum is a pool of human serum that does not contain antinuclear antibodies. This control should give values of less than 10 ANA units.
8. The undiluted positive control serum is a human serum that contains antinuclear antibodies. This control should give a value of greater than 15 ANA units.

INTERPRETATION OF PATIENT RESULTS

This is a qualitative assay. The levels of antibody detected have no known clinical significance, and the Unit values obtained in this assay are designed merely to separate patients into the following three broad groups. Patient sample wells that have calculated values greater than or equal to 15 ANA Units are considered to be positive, and should be tested using a HEp-2 or HEp-2000® slide assay to determine ANA staining patterns, and appropriate follow-up testing. Patient sample wells that have calculated values less than 10 ANA Units are considered to be negative. Values between 10 Units and 15 Units are considered to be borderline positive and should be repeated, or should be tested using a HEp-2 or HEp-2000® slide assay to determine ANA staining patterns, and appropriate follow-up testing. Each laboratory must establish its own reference range and cut-off values based on the population of patients that are being tested. Unit values are affected by patient factors, mechanical considerations (such as pipetting precision and accuracy), and assay conditions (such as temperature and timing of steps.)

REPORTING OF RESULTS

Results should be reported as positive or negative for antinuclear antibodies. The levels of antibodies have no known clinical significance.

Limitations of the Test

1. Diagnosis cannot be made on the basis of antinuclear antibody detection alone. The physician must interpret these results in conjunction with the patient’s history and symptoms, the physical findings, and other diagnostic procedures.
2. Treatment should not be initiated on the sole basis of a positive test for antinuclear antibodies. Clinical indications, other laboratory findings, and the physician’s clinical impression must be considered before any treatment is initiated.
3. Certain drugs, including procainamide and hydralazine, may induce a lupus erythematosus-like disease (22). Patients with drug-induced LE may demonstrate a positive ANA.
4. Although a high-titered ANA may be highly suggestive of connective tissue disease, it should not be considered diagnostic but rather viewed as a part of the overall clinical history of a patient.

5. Positive ANAs are also seen in a small percentage of patients with infectious and/or neoplastic diseases (9).

Expected Values

The incidence of autoantibodies to various nuclear antigens varies depending upon the patient population, and the incidence of clinical rheumatic diseases in that population. Using the indirect immunofluorescent technique with HEp-2 cells, antinuclear antibodies have been detected in 95% of patients with systemic lupus erythematosus, 90% of patients with drug induced lupus, 95% of patients with mixed connective tissue disease, 80% of patients with Sjögren's Syndrome, and 90% of patients with scleroderma (23).

Performance Characteristics

The Immuno Concepts RELISA® ANA Screening Test System was compared to another ELISA antinuclear antibody test kit which is in commercial distribution. The population studied consisted of 579 samples which were submitted to a large University medical center for antinuclear antibody testing, 90 samples which were known positive ANA samples, 242 samples from female blood donors, and 261 samples from male blood donors. All samples were tested in parallel on the predicate device and the subject device. Based on this comparison, the following data were obtained:

		Predicate Device	
IC RELISA® Antinuclear Antibody Test		Positive	Negative
	Positive	214	46
	Borderline	12	105
	Negative	8	787

Borderline results were considered positive. These data show overall agreement of 85.4%.

The large number of "false positive" samples seen with the Immuno Concepts test was troubling, so we tested all of these sera for antinuclear antibodies using Immuno Concepts HEp-2000® ANA-Ro Test System. Seventy-nine of the "false positive" samples were shown to have clearly discernible ANA patterns with the indirect fluorescent antibody technique and were considered "true positives" for the detection of antinuclear antibodies. Four of the "false negative" samples were shown to be negative by the indirect fluorescent antibody technique and were considered "true negatives" for the detection of antinuclear antibodies. Thus, when the reference method is taken into account, the comparison looks like this:

		Reference Method	
IC RELISA® Antinuclear Antibody Test		Positive	Negative
	Positive	305	72
	Negative	4	791

These data show overall agreement of 93.5%.

REPRODUCIBILITY

Nine positive samples, two borderline samples and five negative samples were run on three different lot numbers of antigen coated microwell strips, on multiple occasions by several technologists. In no case did a negative sample show positive results, and the positive samples consistently gave clearly positive results. The borderline samples varied between negative and borderline.

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In the event of damage to the protective packaging, please contact Immuno Concepts prior to use.


Manufacturer

Authorized Representative in the European Community


Temperature Limitation

Contains Sufficient for <n> tests


Consult Instructions for Use

In Vitro Diagnostic Medical Device

MDSS GmbH
Schiffgraben 41
D-30175 Hannover, Germany

Immuno Concepts, N.A. Ltd. 9825 Goethe Road, Suite 350 Sacramento, CA. 95827
Technical Support USA: 1.800.251.5115 Outside USA: 1.916.363.2649
Email: technicalsupport@immunoconcepts.com

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RELISA® ANA TEST PROCEDURE

All samples, reagents (including the wash buffer solution), and microwells must be at room temperature before use.

1. **PREPARE WORKSHEET**
   Label the worksheet that is enclosed in the kit to indicate the location of the samples in the microwells. Assay the calibrator in duplicate. One well is used for a reagent blank. We recommend that each control and patient sample be assayed in duplicate until you have established an acceptable precision for the assay in your laboratory.

2. **PREPARE WASH BUFFER SOLUTION** (PBS-Tween)
   Dissolve contents of one PBS buffer pouch in one liter of deionized or distilled water. Add the entire contents of one bottle of Wash Buffer Concentrate to the one-liter container of dissolved PBS. Mix well. The wash buffer solution may be covered and stored at 2-25°C up to four weeks.

3. **DILUTE PATIENT SAMPLES**
   Dilute patient samples 1:40 by adding 25 µl of serum to 975 µl of Sample Diluent. If you are using the optional ANA undiluted assayed positive control, dilute it in the same manner as the patient samples. Mix well. The calibrator, positive control, and negative control are provided at the working dilution and do not require any further dilution.

4. **PREPARE MICROWELLS**
   Remove the required number of microwell strips from the pouch and place them in the frame holder. The microwells must be firmly seated in the frame holder. Press down firmly on both ends of the strips so that they securely snap into the frame holder. If using individual wells or less than a full strip of wells, be sure each well is firmly seated. Wells that are properly seated in the frame holder will not fall out when the frame holder is inverted. If fewer than eight wells are needed for testing, the wells can be separated by snapping them apart. Unused wells can be returned to the foil pouch, sealed with the zipper seal, and refrigerated for up to 45 days.

5. **DISPENSE SERUM DILUTIONS**
   Dispense 100 µl of the calibrators, controls, and diluted patient samples into the appropriate wells as outlined on the worksheet. Dispense 100 µl of Sample Diluent into the reagent blank well.

6. **INCUBATE STRIPS** (30 minutes at room temperature, i.e. 18-25°C)
   Incubate at room temperature for 30 minutes. The strips should be protected from drafts or shifts in temperature during incubation. If desired, the strips can be covered with transparent tape or a paper towel to protect them from dust or other foreign bodies.

7. **WASH STRIPS** (See General Procedural Notes 5 and 6)
   Wash the wells 3 to 5 times with PBS-Tween Wash Buffer Solution. For manual washing, aspirate the contents of the wells, then fill each well with wash buffer solution. Avoid cross-contamination of the wells, particularly in the first wash after aspiration. Drain all of the wash buffer from the wells by inverting, then shaking residual wash buffer from the wells with a sharp “snapping” motion of the wrist. Repeat the filling and draining steps for a total of 3 to 5 washes. The wells should then be rapped vigorously on a paper towel or other absorbent material to remove all traces of residual wash buffer.

8. **DISPENSE ENZYME ANTIBODY REAGENT**
   Dispense 100 µl of Enzyme Antibody Reagent to each of the wells.

9. **INCUBATE STRIPS** (30 minutes at room temperature, i.e., 18-25°C)
   Incubate at room temperature for 30 minutes. The strips should be protected from drafts or shifts in temperature during incubation. If desired, the strips can be covered with transparent tape or a paper towel to protect them from dust or other foreign bodies.

10. **WASH STRIPS**
    Wash the wells 3 to 5 times with PBS-Tween Wash Buffer Solution. For manual washing, aspirate the contents of the wells, then fill each well with wash buffer solution. Avoid cross-contamination of the wells, particularly in the first wash after aspiration. Drain all of the wash buffer from the wells by inverting, then shaking residual wash buffer from the wells with a sharp “snapping” motion of the wrist. Repeat the filling and draining steps for a total of 3 to 5 washes. The wells should then be rapped vigorously on a paper towel or other absorbent material to remove all traces of residual wash buffer.

11. **DISPENSE SUBSTRATE SOLUTION**
    Using a timer to assure consistent intervals, dispense 100 µl of Substrate Solution to each of the wells. The Substrate Solution must be added to the wells at a steady rate, so that each well is incubated for exactly the same length of time (30 minutes). The substrate solution in wells incubated with positive samples will turn blue, and the solution in wells incubated with negative samples will be colorless to very pale blue.

12. **INCUBATE STRIPS** (Exactly 30 minutes at room temperature, i.e., 18-25°C)
    Incubate at room temperature for exactly 30 minutes. The strips should be protected from drafts or shifts in temperature during incubation.

13. **DISPENSE STOPPING REAGENT**
    After the first well has incubated for exactly 30 minutes, add 100 µl of Stopping Reagent to each well, in the same order and at the same rate that the Substrate Solution was added to the wells. Upon addition of stopping reagent, blue substrate solution will turn yellow and colorless solution will remain colorless.

14. **READ ABSORBANCE OF WELLS**
    Within 30 minutes after addition of the stopping reagent, the wells must be read in a plate reading spectrophotometer. The wells are read at 450 nm against the blank control well. If a dual wavelength spectrophotometer is available, the wavelength for the reference filter should be set at 600-650 nm. Reading the microwells at 450 nm without a reference filter will result in higher absorbance values.

FOR TECHNICAL ASSISTANCE:
USA: 1-800-251-5115 Outside USA: 1-916-363-2649
Email: technicalsupport@immunoconcepts.com