Trinity Biotech

Captia™ ENA Profile

REF 2338170 12 Tests

Pour d'autres langues	Para outras línguas
Für andere Sprachen	Για τις άλλες λώσσες
Para otras lenguas	För andra språk
Per le altre lingue	For andre språk
Dla innych j@zyków	Pro jiné jazyky

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INTENDED USE

The Trinity Biotech Captia™ ENA Profile Enzyme-Linked Immunosorbent Assay (ELISA) is intended for the detection of antibodies in human serum to individual antigens for Smith, Sm/RNP, SS-A (Ro), SS-B (La), Scl-70 and Jo-1 in a single serum specimen. The results of the assay are to be used as an aid in the diagnosis of Systemic Lupus Erthematosus (SLE), scleroderma, Sjogren's syndrome (SS), or other systemic rheumatic diseases. **For *in vitro* diagnostic use. High complexity test.**

INTRODUCTION

Autoimmune diseases are categorized into two general groups: One group is characterized by the production of tissue-specific autoantibodies, the other by autoimmune reactivity with normal cell nuclear and/or cytoplasmic antigens with no tissue type specificity. The latter group includes diseases such as scleroderma, rheumatoid arthritis, dermatomyositis, polymyositis, Sjogren's syndrome, and systemic lupus erythematosus (SLE).[1]

The non-histone Smith (Sm) antigen occurs in close association with the RNP antigen, in multimolecular complexes. They are small nuclear riboproteins consisting of uridine-rich U series small nuclear RNAs. The Sm antigen is comprised of U 1, U 2, U 4, U 5, and U 6 RNA and RNP is comprised of U 1 RNA.[2]

Approximately 20-40% of SLE patients show the presence of Sm antibody. This is highly indicative of SLE because anti-Sm alone is not found in normal individuals or in patients with other systemic rheumatic diseases. Anti-RNP is present in a variety of systemic rheumatic diseases, including Sjogren's syndrome, rheumatoid arthritis, and SLE. A high titer of anti-RNP, in the absence of other ANAs, strongly suggests mixed connective tissue disease (MCTD).[3,4,5,6,7,8] The RNP antigen is commonly found as an Sm/RNP protein complex, therefore, a positive patient result should be run in an anti-Sm test as an additional aid in the diagnosis of SLE. Only when RNP antigen has been separated from the Sm/RNP complex can it be considered as an accurate marker for mixed connective tissue disease.

Sjogren's syndrome is a chronic inflammatory autoimmune disease characterized by decreased lachrymal and salivary gland secretion resulting in dry eye syndrome (keratoconjunctivitis sicca) and dryness of the mouth (xerostomia). Sjogren's syndrome is found primarily in females (90%) and may be explained by the influence of sex steroid hormones on autoimmunity and immunoregulation. Histological evidence associates the presence of SS-A and SS-B antibodies with the presence of Sjogren's syndrome.[9] There are several associations between anti- SS-A/Ro antibody and certain systemic rheumatic diseases. This antibody is found in 60-70% of patients with Sjogren's syndrome and 30- 40% of patients with SLE. Antibody to SS-B/La antigen is detected in approximately 15% of SLE sera. It is detected in a higher percentage (60%) of sera of patients with SS.[3]

Scleroderma, also known as progressive systemic sclerosis is a connective tissue autoimmune disease of unknown etiology in which thickening of the skin is the primary feature, often accompanied by smooth muscle atrophy, and fibrosis of internal organs such as the lungs, heart and intestines. Two sub-types of scleroderma have been identified and classified as: 1) diffuse or generalized scleroderma, and 2) a more limited form known as CREST syndrome. Antibodies to the antigen Scl- 70, a 70,000 dalton nonhistone nuclear protein known as DNA topoisomerase 1[10,11] are found in approximately 20% of patients with scleroderma and are seldom seen in patients with other autoimmune diseases. Therefore this antibody appears to be extremely specific for scleroderma, especially the diffuse form,[12] and is not found in Systemic lupus erythematosus, Sjogren's or other rheumatoid syndromes.

The inflammatory myopathies are a group of diseases in which the muscles are diffusely damaged by a perivascular and/or interstitial infiltration of inflammatory cells, with predominantly lymphocytes. The clinical syndrome associated with these pathological changes is termed polymyositis.[13] Autoantibodies to the extractable cytoplasmic antigen Jo-1 (histidyl- tRNA ligase) have been reported in 18-36% of patients with polymyositis, an inflammatory autoimmune disease of skeletal muscle. These antibodies are more common in polymyositis than in dermatomyositis, a cutaneous variant of polymyositis, and are rarely found in other diseases. Autoantibodies to Jo-1 are strongly associated with interstitial lung disease in polymyositis patients, and nearly all anti-Jo-1 positive patients show clinical or radiographic evidence of lung involvement. The Jo-1 antigen is one of a family of enzymes that catalyze the attachment of amino acids to their cognate tRNA molecules.[14]

Tests for the detection of antibodies to the ENA marker antigens use standard immunologic techniques, including immunodiffusion, counterimmunoelectrophoresis, immunofluorescence, and hemagglutination. These tests require long incubation times and experienced technicians to perform the assays which produce subjective readings. Enzyme immunoassays are more sensitive with minimal technician training and yield ojective results.

PRINCIPLE OF THE ASSAY

The Trinity Biotech ENA Profile test is an Enzyme-Linked Immunosorbent Assay to detect IgG, IgA, and IgM antibodies to antigens identified as Smith (Sm), Sm/RNP (ribonucleoprotein), SS-A (Ro), SS-B (La), Scl-70 and Jo-1.The purified antigens are attached to a solid phase microassay well. Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG,A,M conjugated with horseradish peroxidase which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H_2SO_4, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.[20,21,22,23]

KIT PRESENTATION

MATERIALS SUPPLIED

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on the package label.

1. Purified Sm, Sm/RNP, SS-A, SS-B, Scl-70 and Jo-1 antigen coated microassay plate: 96 wells, configured in twelve 1 X 8 strips stored in a foil pouch with desiccant. (12 T: one plate, each eight well strip is used to perform one ENA Profile test)
2. Serum Diluent Type III: Ready to use. Contains buffer, BSA and Tween-20, and ProClin® (0.1%) as a preservative. (96T: one bottle, 30 mL)
3. Calibrator: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with kit specific factor printed on vial label. The Calibrator is used to calibrate the assay to account for day-to-day fluctuations in temperature and other testing conditions. (96T: one vial, 0.4 mL) *
4. Negative Control: Human serum or defibrinated plasma. Sodium azide (< 0.1%) and pen/strep (0.01%) added as preservatives, with established range printed on vial label. The Negative Control is utilized to control the negative range of the assay. (96T: one vial, 0.4 mL) *
5. Horseradish-peroxidase (HRP) Conjugate: Ready to use. Goat anti-human IgG, IgA, and IgM containing ProClin® (0.1%) and gentamicin as preservatives. (96T: one bottle, 15 mL)
6. Wash Buffer Type II (20X concentrate): Dilute 1 part concentrate + 19 parts deionized or distilled water. Contains TBS, Tween-80 and ProClin® (0.1%) as a preservative. (96T: one bottle, 50 mL)
7. Chromogen/Substrate Solution Type I: Tetramethylbenzidine (TMB), ready to use. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. (96T: one bottle, 15 mL)
8. Stop Solution: Ready to use, contains a 1N H_2SO_4 solution. (96T: one bottle, 15 mL)
9. Blank strip: 1X8 well strip, ready to be used as reagent blank.

*** Note: serum vials may contain excess volume.**

The following components are not kit lot # dependent and may be used interchangeably within the Trinity Biotech ELISA Autoimmune Kits: Serum Diluent Type III, Chromogen/Substrate Solution Type I, Wash Buffer Type II, and Stop Solution. Please check that the appropriate Trinity Biotech Reagent Type (Type I, Type II, etc.) is used for the assay.

ADDITIONAL REQUIREMENTS

- Wash bottle, automated or semi-automated microwell plate washing system.
- Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes (less than 3% CV).
- One liter graduated cylinder.
- Paper towels.
- Test tube for serum dilution.
- Reagent reservoirs for multichannel pipettes.
- Pipette tips.
- Distilled or deionized water (dH_20), CAP (College of American Pathology) Type 1 or equivalent.[17,18]
- Timer capable of measuring to an accuracy of +/- 1 second (0 - 60 minutes).
- Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH_20).
- Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Note: Use only clean, dry glassware.

STORAGE AND STABILITY

1. Store unopened kit between 2° and 8° C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2° and 8° C. Unused strips must be immediately resealed in a sealable bag with desiccant, and returned to storage at 2° and 8° C. If the bag is resealed with tape, the wells are stable for 30 days. If the bag is resealed with a heat sealer, the wells are stable until their labeled expiration date.
3. Store HRP Conjugate between 2° and 8° C.
4. Store the Calibrator, High Positive, Low Positive and Negative Controls between 2° and 8° C.
5. Store Serum Diluent Type III and 20X Wash Buffer Type II between 2° and 8° C.
6. Store the Chromogen/Substrate Solution Type I between 2° and 8° C. The reagent should remain closed when not in use. If allowed to evaporate, a precipitate may form in the reagent wells. 7. Store 1X (diluted) Wash Buffer Type II at room temperature (21° to 25° C) for up to 5 days, or up to 1 week between 2° and 8° C.

Note: If constant storage temperature is maintained, reagents and substrate will be stable for the dating period of the kit. Refer to package label for expiration date. Precautions were taken in the manufacture of this product to protect the reagents from contamination and bacteriostatic agents have been added to the liquid reagents. Care should be exercised to protect the reagents in this kit from contamination. Do not use if evidence of microbial contamination or precipitation is present.

PRECAUTIONS

1. For *in vitro* diagnostic use.
2. The human serum components used in the preparation of the Controls and Calibrator in this kit have been tested by an FDA approved method for the presence of antibodies to human immunodeficiency virus 1 & 2 (HIV 1&2), hepatitis C (HCV) as well as hepatitis B surface antigen and found negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, specimens and human-based reagents should be handled as if capable of transmitting infectious agents.
3. The Centers for Disease Control & Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.[10]
4. The components in this kit have been quality control tested as a Master Lot unit. Do not mix components from different lot numbers except Chromogen/Substrate Solution Type I, Stop Solution, Wash Buffer Type II, and Serum Diluent Type III. Do not mix with components from other manufacturers.
5. Do not use reagents beyond the stated expiration date marked on the package label.
6. All reagents must be at room temperature (21°to 25°C) before running assay. Remove only the volume of reagents that is needed. **Do not pour reagents back into vials as reagent contamination may occur.**
7. Before opening Control and Calibrator vials, tap firmly on the benchtop to ensure that all liquid is at the bottom of the vial.
8. Use only distilled or deionized water and clean glassware.
9. Do not let wells dry during assay; add reagents immediately after completing wash steps.
10. Avoid cross-contamination of reagents. Avoid splashing or generation of aerosols. Wash hands before and after handling reagents. **Cross-contamination of reagents and/or samples could cause erroneous results.**
11. If washing steps are performed manually, wells are to be washed three times. Up to five wash cycles may be necessary if a washing manifold or automated equipment is used.
12. **Sodium azide inhibits Conjugate activity. Clean pipette tips must be used for the Conjugate addition so that sodium azide is not carried over from other reagents.**
13. Certain reagents in this kit contain sodium azide for use as a preservative. It has been reported that sodium azide may react with lead and copper in plumbing to form explosive compounds. When disposing, flush drains with water to minimize build-up of metal azide compounds.
14. Never pipette by mouth or allow reagents or patient sample to come into contact with skin. Reagents containing ProClin®, sodium azide, and TMB may be irritating. Avoid contact with skin and eyes. In case of contact, immediately flush area with copious amounts of water.
15. If a sodium hypochlorite (bleach) solution is being used as a disinfectant, do not expose to work area during actual test procedure because of potential interference with enzyme activity.
16. Avoid contact of Stop Solution (1N sulfuric acid) with skin or eyes. If contact occurs, immediately flush area with copious amounts of water.
17. **Caution:** Liquid waste at acid pH must be neutralized prior to adding sodium hypochlorite (bleach) solution to avoid formation of poisonous gas. Recommend disposing of reacted, stopped plates in biohazard bags. See Precaution 3.
18. Do not use Chromogen/Substrate Solution if it has begun to turn blue.
19. The concentrations of anti-extractable nuclear antigens (Sm, SM/RNP, SS-A, SS-B, Scl-70 and Jo-1) in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

The safety data sheet is available upon request.

WARNING

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

P280: Wear protective gloves / protective clothing / eye protection / face protection.

P302 + **P352**: IF ON SKIN: Wash with plenty of soap and water.

P333 + **P313**: If skin irritation or rash occurs: Get medical advice/ attention.

P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Serum Diluent and Controls contain < 0.1% sodium azide.

H302: Harmful if swallowed

P264: Wash thoroughly with plenty of soap and water after handling

P270: Do not eat, drink or smoke when using this product

P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell

P330: If swallowed, rinse mouth

P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

SPECIMEN COLLECTION AND STORAGE

1. Handle all blood and serum as if capable of transmitting infectious agents.[10]
2. Optimal performance of the Trinity Biotech ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, nonlipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture.[11] Early separation from the clot prevents hemolysis of serum.
3. Store serum between 2° and 8° C if testing will take place within five days. If specimens are to be kept for longer periods, store at -20° C to -70° C in a non-defrosting freezer. Do not use a frostfree freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. Serum containing visible particulate matter can be spun down utilizing slow speed centrifugation.
5. Do not use heat inactivate sera.
6. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).[11]

METHODS FOR USE

PREPARATION FOR THE ASSAY

1. All reagents must be removed from refrigeration and allowed to come to room temperature before use (21° to 25° C). Return all reagents to refrigerator promptly after use.
2. All samples and controls should be vortexed before use.
3. Dilute 50 mL of the 20X Wash Buffer Type II to 1L with distilled and/or deionized H_20. Mix well.

ASSAY PROCEDURE

1. Determine the number of patients to be assayed. Provide one 8-well strip for each patient sample to be run: (Negative Control well (well "A"), Calibrator well (well "B"), patient sera wells (wells "C" through "H")). Check ENA Profile Worksheet Plate Format for the correct Control/Calibrator and patient sample configurations.

Example Configuration:

Plate Location	Sample Description	Plate Location	Sample Description
1A	Neg Control	2A	Neg Control
1B	Calibrator	2B	Calibrator
1C	Patient #1 (RNP)	2C	Patient #2 (RNP)
1D	Patient #1 (Sm)	2D	Patient #2 (Sm)
1E	Patient #1 (Jo-1)	2E	Patient #2 (Jo-1)
1F	Patient #1 (Scl-70)	2F	Patient #2 (Scl-70)
1G	Patient #1 (SS-A)	2G	Patient #2 (SS-A)
1H	Patient #1 (SS-B)	2H	Patient #2 (SS-B)

2. Dilute test sera, Calibrator and Control sera 1:21 (e.g., 10 µL + 200 µL) in Serum Diluent. A total of 800 µL is needed for each patient sample; therefore it is recommended to make one dilution of 40 µL (patient sample) + 800 µL (Serum Diluent). Dilute Negative Control and Calibrator 10 µL + 200 µL (Serum Diluent). Mix well. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the patient serum.)
3. Add 100 µL of diluted Negative Control into well "A" of each strip and 100 µL of diluted Calibrator into well "B" of each strip. Pipette 100 µL of patient sample into wells "C" through "H", using one stip per patient. Add 100 µL of Serum Diluent to reagent blank well, using one well from blank strip provided. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21° to 25° C) for **30 minutes +/- 1 minute**.
5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four times (for a total of five (5) washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

****IMPORTANT NOTE:** Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 L) is recommended. A total of up to five (5) washes may be necessary with automated equipment. **Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.**

6. Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21° to 25° C) for **30 minutes +/- 1 minute**.
8. Repeat wash as described in Step 5.
9. Add 100 µL Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21° to 25° C) for **15 minutes +/- 1 minute**.
11. Stop reaction by addition of 100 µL of Stop Solution (1N H_2SO_4) following the same order of Chromogen/Substrate addition, including the reagent blank well.Tap the plate gently along the outsides, to mix contents of the wells. The plate may be held up to 1 hour after addition of the Stop Solution before reading.
12. The developed color should be read on an ELISA plate reader equipped with a 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. The instrument should be blanked on air. The reagent blank must be less than 0.150 Absorbance at 450 nm. If the reagent blank is ≥ 0.150 the run must be repeated. Blank the reader on the reagent blank well and then continue to read the entire plate. Dispose of used plates after readings have been obtained.

QUALITY CONTROL

For the assay to be considered valid the following conditions must be met:

1. Calibrators and Negative Control must be run with each test run.
2. Reagent Blank must be < 0.150 O.D. (Optical Density) at 450 nm (when read against Air Blank).
3. The mean O.D. value for the Calibrator should be ≥ 0.300 at 450 nm (when read against Reagent Blank).
4. The Index Values for the Negative Control should be calculated using the Sm/RNP correction factor. The Index Value for the Negative Control should be in its range printed on the vial label. If the Negative Control value is not within its range, the test should be considered invalid and should be repeated.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to NCCLS C24A for guidance on appropriate Quality Control practices.[14]
7. If above criteria are not met on repeat, contact Trinity Biotech Technical Service.

INTERPRETATION

1. Correction Factor - To account for day-to-day fluctuations in assay activity due to room temperature and timing, antigen specific Correction Factors are determined by Trinity Biotech for each lot of kits and are printed on the component label.
2. Cutoff O.D. Value - The Cutoff O.D. Value for each antigen is determined by multiplying the antigen specific Correction Factor by the mean Calibrator absorbance. This must be done for each individual antigen.
3. Index Value - Calculate an Index Value for each antigen of all patient samples by dividing the patient sample O.D. value obtained for each antigen by the specific Cutoff O.D. determined in Step 2.

Example :

Absorbance of Calibrator (well B)	= 0.400
O.D. obtained for patient sample In Sm (well D)	= 0.600
Sm specific Correction Factor	= 0.25
Sm Cutoff Value	= 0.25 x 0.400 = 0.100
Sm Index Value	= 0.600/0.100 = 6.00
O.D. obtained for patient sample In SS-A (well G)	= 0.050
SS-A specific Correction factor	= 0.30
Sm Cutoff Value	= 0.30 x 0.400 = 0.120
Sm Index Value	= .050/0.120 = 0.42

ANALYSIS

1. The patients' Index Values are interpreted as follows:

Index Value	Results	Interpretation
≤. 0.90	Negative	No detectable antibody to Extractable Nuclear Antigens by the ELISA test.
0.91-1.09	Equivocal	Samples should be re-tested. See number 2 below.
≥ 1.10	Positive	Indicates presence of detectable antibody to Extractable Nuclear Antigens by the ELISA test.

2. Samples that remain equivocal after repeat testing should be retested on an alternate method or test a new sample.

EXPECTED VALUES

Autoimmune rheumatic diseases are a group of chronic disorders, afflicting about 3% of the population. The etiology of these disorders is not elucidated but they may involve both genetic and environmental causes. Autoimmune rheumatic diseases have two common clinical and pathological features: A) a non-organ specific autoimmune condition is manifest; B) most patients exhibit some rheumatic symptoms throughout the course of their disease. It is apparent that certain systemic rheumatic diseases have distinct ANA profiles. Thus the ANA profile is helpful in the evaluation of patients with systemic lupus erythematosus, mixed connective tissue disease, scleroderma, Sjogrens's syndrome, dermatopolymyositis, and rheumatoid arthritis (RA). Antibody to Sm/RNP antigen is found in approximately 25 - 30% of patients with SLE. This antibody is considered a highly specific marker for patients with SLE because it has not been detected in normal individuals or in patients with other systemic rheumatic diseases. Anti-RNP is present in a variety of systemic rheumatic diseases, including Sjogren's syndrome, rheumatoid arthritis, and SLE. A high titer of anti-RNP, in the absence of other ANAs, strongly suggests mixed connective tissue disease[3] if RNP is separated from the Sm/RMP complex.

The following chart below shows some reported incidences of the ENA antibodies in systemic lupus erythematosus (SLE), Sjogren's syndrome (SS), progressive systemic sclerosis (PSS), polymyositis (PM), dermatomyositis (DM), mixed connective tissue disease (MCTD):

Disease Antibody	SLE	SS	PSS	PM/DM	MCTD
Sm	25-40%	-	-	-	0-5%
RNP	25-50%	0-5%	20-25%	-	95-100%
SS-A	30-40%	35-70%	-	-	-
SS-B	10-15%	50-60%	-	-	-
Scl-70	-	0-10%	15-30%	-	-
Jo-1	-	-	-	20-30%	-

Data compiled from literature.[13,14,15]

LIMITATIONS OF USE

1. Only if test instructions are rigidly followed will optimum results be achieved.
2. Reproducible results depend on careful pipetting, observation of incubation periods and temperature, as well as washing the test strips and thorough mixing of all prepared solutions.
3. If comparisons with other methods are required, always perform both tests simultaneously.
4. Do not scratch coated wells during washing and aspiration. Wash and fill all reagents without interruption. While washing, check that all wells are filled evenly with washing solution, and that there are no residues in the wells.
5. Instructions for using appropriate photometers are to be observed; check adjustment of proper wave length (450 nm) and reference wave length (600-650 nm) (optional) respectively.
6. The values obtained from this assay are intended to be an aid to diagnosis only. Each physician must interpret the results in conjunction with the patient's history, physical findings and other diagnostic procedures.
7. This test is not intended for the determination of immune status as related to immunity. Immune status cannot be determined by this assay and is intended for the detection of antibody to Sm, Sm/RNP, SS-A, SS-B, Scl-70 and Jo-1 antigens.
8. The Sm/RNP antigen used in this assay is a protein complex, therefore, a positive patient result in the Sm/RNP well should be read in relation to the result of the Sm well. Only when RNP antigen has been separated from the Sm/RNP complex can it be considered as an accurate marker for mixed connective tissue disease. To obtain an RNP only value, the Index Value obtained in the Sm well should be subtracted from the Sm/RNP Index Value.
9. Sera from patients with other autoimmune disease and from normal individuals may contain autoantibodies.
10. The assay should be used only with serum. Icteric, lipemic, hemolyzed, and heat inactivated serum should be avoided.

PERFORMANCE CHARACTERISTICS

RELATIVE SENSITIVITY AND SPECIFICITY

Frozen retrospective normal sera and sera from autoimmune patients were tested on the Trinity Biotech ENA Profile, Trinity Biotech's individual ENA's and commercially available individual ENA kits. 43 normal sera were tested for each antigen. Sera from selected known lupus patients were assayed on Sm, Sm/RNP, SS-A, and SS-B kits. Selected sera positive for Jo-1 and Scl-70 were assayed on Scl-70 and Jo-1 kits. The relative sensitivity and specificity data was compiled for each individual antigen. The results are summarized in the following tables. All equivocals were excluded from the calculations.

Trinity Biotech Individual ENA Assays

Antigen	Number of sera tested	Relative Sensitivity	Relative Specificity
Sm	88	100% (26/26)	98.4% (54/56)
Sm/RNP	88	100% (45/45)	100% (43/43)
SS-A	88	97.7% (43/44)	100% (44/44)
SS-B	75	96.7% (29/30)	100% (44/44)
Scl-70	57	100% (13/13)	100% (43/43)
Jo-1	57	100% (13/13/)	100% (43/43)

Commercially Available Individual ENA Assays

Antigen	Number of sera tested	Relative Sensitivity	Relative Specificity
Sm	88	83.9% (26/31)	96.1% (49/51)
Sm/RNP	88	100% (45/45)	100% (43/43)
SS-A	88	100% (43/43)	100% (43/43)
SS-B	75	93.5% (29/31)	100% (44/44)
Scl-70	57	100% (14/14)	100% (43/43)
Jo-1	57	100% (13/13/)	100% (43/43)

REPRODUCIBILITY

Six different sera (one positive for each antigen) were assayed to determine the reproducibility of the Trinity Biotech ENA Profile ELISA assay. Each serum was tested eight times each, on three different assays. The intra-assay and inter-assay coefficient of variation (CV) for each serum is presented in the following table.

Reproducibility Study

	Assay 1 (n=8)			Assay 2 (n=8)			Assay 3 (n=8)			Inter Assay (n=24)		
Sera #	X	S.D.	CV%	X	S.D.	CV%	X	S.D.	CV%	X	S.D.	CV%
Sm	1.80	0.149	8.27%	2.02	0.165	8.19%	1.39	0.137	8.15%	1.84	0.201	10.94%
Sm/RNP	1.80	0.164	9.09%	1.79	0.155	8.62%	1.59	0.126	7.93%	1.73	0.173	10.01%
SS-A	2.78	0.122	4.38%	2.91	0.242	8.32%	2.61	0.205	7.84%	2.77	0.225	8.13%
SS-B	2.71	0.142	5.25%	2.88	0.165	5.71%	2.73	0.201	7.37%	2.77	0.182	6.56%
Scl-70	2.39	0.249	10.41%	2.41	0.189	7.72%	2.17	0.087	4.00%	2.32	0.212	9.13%
Jo-1	2.23	0.229	10.28%	2.39	0.358	14.96%	2.16	0.323	14.94%	2.26	0.311	13.73%

LINEARITY

The Index Values were determined for serial two fold dilutions of positive sera for each antigen. The Index Values were compared to $\log_2$ of dilution by standard linear regression. The data in the following table indicates that the assay has a linear relationship with serum dilution.

Linearity

Serum #	Neat	1:2	1:4	1:8	1:16	1:32	1:64	r^2
Sm	9.10	7.56	6.68	4.43	2.93	1.50	1.17	0.988
Sm/RNP	6.70	5.19	4.06	3.05	1.91	1.00	0.50	0.992
SS-A	5.73	5.28	4.43	4.08	3.10	2.28	1.43	0.994
SS-B	9.21	7.92	6.44	4.38	3.23	1.21	0.46	0.996
Scl-70	5.82	3.53	1.87	1.02	0.55			0.961
Jo-1	7.44	5.97	4.08	2.46	1.10	0.72		0.987

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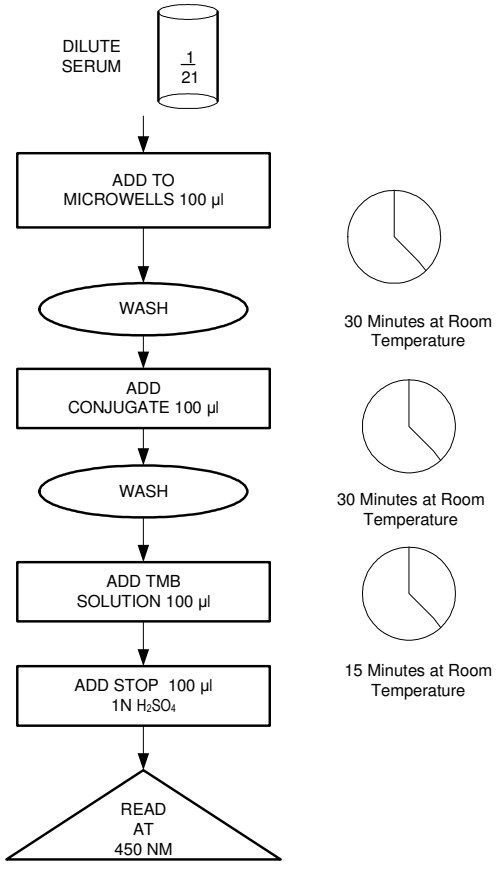
CAPTIA™ ENA PROFILE SUMMARY OF ASSAY PROCEDURE



The safety data sheet is available upon request.

WARNING

Serum Diluent, Conjugate, and Wash Buffer contain 0.1% ProClin 300®, a biocidal preservative that may cause sensitization by skin contact; prolonged or repeated exposure may cause allergic reaction in certain sensitive individuals.

H317: May cause an allergic skin reaction.

P280: Wear protective gloves / protective clothing / eye protection / face protection.

P302 + P352: IF ON SKIN: Wash with plenty of soap and water.

P333 + P313: If skin irritation or rash occurs: Get medical advice/ attention.

P501: Dispose of contents and container in accordance to local, regional, national and international regulations.

WARNING

Serum Diluent and Controls contain < 0.1% sodium azide.

H302: Harmful if swallowed

P264: Wash thoroughly with plenty of soap and water after handling

P270: Do not eat, drink or smoke when using this product

P301+P312: IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell

P330: If swallowed, rinse mouth

P501: Dispose of contents/container to in accordance to local, regional, national and international regulations.

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Catalog No.	Item	Quantity
2338170	Captia™ ENA Profile Test Kit	12 Tests

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EC REP
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Consult accompanying documents

REF
Product Number

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Caution, consult accompanying documents

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CONTROL + +
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CONTROL +
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