ReQuest® ENA PLUS SCREENING

REF 219350 96-Test Set IVD

Intended Use: For the qualitative screening of human antibodies to Extractable Nuclear Antigens (ENA) in human serum by enzyme immunoassay, to aid in the diagnosis of certain systematic rheumatic diseases. This assay collectively detects in one well, SS-A/Ro, SS-B/La, Sm, Sm/RNP, Scl-70 and Jo-1. This assay has not been cleared / approved by the FDA for blood / plasma donor screening.

For in vitro diagnostic use only

Summary of Test

1. Prepare 1:40 dilutions of Controls and samples in the SQ-ENA Diluent. Mix well.
2. Place 100 µl of the dilutions in the SQ-ENA Wells; reserve one well for the reagent blank.
3. Incubate at room temperature for 30 ± 5 minutes.
4. Drain wells thoroughly. Wash wells 5 times with diluted SQ-ENA Wash solution and drain.
5. Place 100 µl of SQ-ENA Conjugate in wells.
6. Incubate at room temperature for 30 ± 5 minutes.
7. Drain wells thoroughly. Wash wells 5 times with diluted SQ-ENA Wash Solution and drain.
8. Place 100 µl of SQ-ENA Substrate in wells.
9. Incubate at room temperature for 30 ± 5 minutes.
10. Stop the enzyme reaction with 100 µl of SQ-ENA Stop.
11. Read absorbance at 450 nm against reagent blank.

Summary and Explanation of Test

Antinuclear antibodies (ANAs) directed against a variety of macromolecules occur in extraordinarily high frequency in systemic rheumatic diseases. Many rheumatic diseases are characterized by the presence of one or more of these antinuclear antibodies. Therefore, the identification of the specific antibody is useful in the detection and diagnosis of the disease (1).
Extractable nuclear antigens (ENAs) are acidic (non-histone) macromolecules extracted from the saline soluble fraction of cell nuclei; hence the term ENA (2). There are now 20 different saline-extractable antigens identified (2). Antibodies to ENAs are seen in several clinical syndromes including Systemic Lupus Erythematosus (SLE), Mixed Connective Tissue Disease (MTCD), Sjogrens Syndrome (SS), Myositis and Progressive Systemic Sclerosis (PSS) and are considered to be markers for these diseases (2-3).

The ReQuest® ENA PLUS SCREENING test simultaneously screens in a single well for SS-A/Ro, SS-B/La, Sm, RNP, Scl-70, and Jo-1 autoantibodies. Samples found to be negative can be considered not positive for SS-A/Ro, SS-B/La, Sm, RNP, Scl-70 and Jo-1.

Principle of the Test

Diluted samples are incubated in antigen-coated wells. ENAs IgG antibodies (if present) are immobilized in the wells. Residual sample is eliminated by washing and draining, and conjugate (enzyme labeled antibodies to human IgG) is added and incubated. If IgG antibodies to ENA are present, the conjugate will be immobilized in the wells. Residual conjugate is eliminated by washing and draining, and the substrate is added and incubated. In the presence of the enzyme, the substrate is converted to a yellow end product which is read photometrically.

Reagents

Component	Content
01-311 Coated Wells	Plastic Microwells: Coated with purified nuclear antigens.
01-312 Conjugate	Goat Anti-Human immunoglobulin labeled with HRP.
01-313 Negative Control	Normal human serum. - Preserved **with ≤20% Glycerol [C3H8O3], CAS# 56-81-5, EC No 200-289-5 [Not subject to GHS and EU 2008/1272/EC regulatory requirements.]**
01-314 Cut-Off Control	Normal human serum. - Preserved **with ≤20% Glycerol [C3H8O3], CAS# 56-81-5, EC No 200-289-5 [Not subject to GHS and EU 2008/1272/EC regulatory requirements.]**
01-315 Positive Control	Normal human serum. - Preserved **with ≤20% Glycerol [C3H8O3], CAS# 56-81-5, EC No 200-289-5 [Not subject to GHS and EU 2008/1272/EC regulatory requirements.]**
01-316 Sample Diluent	Saline with a protein stabilizer.
01-317 Substrate	**TMB (3,3′,5,5′-Tetramethylbenzidine) CAS#*54827-17-7***
01-318 Wash Concentrate	**Phosphate-buffered saline** with **Tween 20**, pH 8.0. Not subject to GHS, US HCS, EC CLP, and analogous global GHS-based regulatory requirements in this product mixture and concentration.
01-319 Stop Solution	**Sulfuric Acid CAS#** 7664-93-9

* Contains sodium azide.

Store these reagents according to the instructions on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water.

Other Materials Required

1. Wash bottle
2. Pipettors for dispensing 10, 100 µl and 400 µl
3. Timer
4. 1 or 2 liter container for Wash Solution

L-01-330H-2
2017-06

CE

5. Distilled or deionized water
6. Dilution tubes or microwells
7. Microwell reader capable of reading absorbance at 450 nm

Precautions

1. For in vitro diagnostic use.
2. Test samples, Controls and the materials that contact them, should be handled as potential biohazards. The controls have been found to be negative for HIV, hepatitis B surface antigen and HCV antibodies by licensed tests. However, because no method can offer complete assurance that HIV, hepatitis B virus, HCV or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual "*Biosafety in Microbiological and Biomedical Laboratories*", 1993, or latest edition.
3. Avoid contact with open skin.
4. Never pipet by mouth.
5. Certain of the test reagents contain sodium azide. Azides are reported to react with lead and copper in plumbing to form compounds that may detonate on percussion. When disposing of solutions containing sodium azide, flush drains with large volumes of water to minimize the build-up of metal-azide compounds.
   R 20/21/22: Harmful by inhalation, in contact with skin and if swallowed.
   S36/37: Wear suitable protective clothing and gloves.
6. SQ-ENA Stop contains a dilute acid solution. Use with care to avoid contact with skin and eyes. Avoid exposure to bases, metals or other compounds which may react with acids. Spills should be cleaned up immediately.
7. Do not interchange reagents from different reagent lots, except for SQ-ENA Wash, SQ-ENA Substrate, and SQ-ENA Stop.
8. Do not use reagents beyond their stated expiration date.
9. Incubation times recommended in the Test Procedure section should be adhered to.
10. Unused SQ-ENA IgG Wells should be kept in their resealable bag with desiccant, and stored in the refrigerator.
11. This product should be used by qualified personnel.

Specimen Collection

Sera should be separated from clotted blood. If specimens are not tested within 8 hours, they should be stored at 2 to 8ºC for up to 24 hours. Beyond 24 hours specimens should be stored at -20ºC or below. Multiple freeze-thaw cycles should be avoided. Samples containing visible particulate matter should be clarified by centrifugation; and hemolyzed, icteric or grossly contaminated samples should not be used. Samples should not be heat-inactivated before testing.

Test Procedure

Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use. The test procedure follows:

1. Prepare 1:40 dilutions of test samples and Controls, in the SQ-ENA Diluent. For example: add 10 µl of sample to 400 µl of SQ-ENA Diluent in a dilution well or tube, and mix well.
2. Place an appropriate number of coated wells from SQ-ENA IgG Wells in the Well Support.
3. Transfer 100 µl of each diluted Controls and patient sample to the wells.
   Note: Include one well which contains 100 µl of SQ-ENA Diluent only. This will serve as the reagent blank and will be ultimately used to zero the photometer before reading the test results.
4. Incubate the wells at room temperature (20 to 25ºC) for 30 ± 5 minutes.
5. Wash wells five times with diluted SQ-ENA Wash solution, drain thoroughly.
6. Place 100 µl of SQ-ENA IgG Conjugate into each well.
7. Incubate the wells at room temperature (20 to 25ºC) for 30 ± 5 minutes.
8. Wash the wells five times with diluted SQ-ENA Wash solution, drain thoroughly.
9. Place 100 µl of SQ-ENA Substrate into each well.
10. Incubate at room temperature (20 to 25ºC) for 30 ± 5 minutes.
11. Place 100 µl of SQ-ENA Stop into each well.
12. Read and record the absorbance of the contents of each well at 450 nm against the reagent blank.
    Note: Adjust the photometer to zero absorbance at 450 nm against the reagent blank. Readings should be made within 2 hours after the reactions have been stopped.

Calculation of Results

Determine the Enzyme Units (EUs)* for each patient specimen (or control) using the following formula

$$\frac{\text{EU of SQ-ENA Cut-off Control}}{\text{SQ-ENA Cut-off Control Absorbance}} \times \text{Test sample absorbance} = \text{EU of Test Sample*}$$

Optional Index calculation: Divide the EU of test sample by 25 to obtain the Index Value*.

*Enzyme Units (EUs) and Index Values are semi-quantitative. They are units defined by ReQuest®.

If the Cut off control is run in duplicate, use the average absorbance value to calculate results.

Quality Control

1. The Controls, SQ-ENA IgG Positive Control, SQ-ENA IgG Cut-off Control and SQ-ENA IgG Negative Control and SQ-ENA Diluent must be included in each test run.
2. The absorbance value of the reagent blank should be less than 0.200.
3. The SQ-ENA IgG Positive Control and SQ-ENA IgG Negative Control must have an absorbance values within the specified range printed on the quality control card included with each kit lot number.
   If any of these criteria are not met, the test is invalid and should be repeated.

L-01-330H-2
2017-06

CE

Interpretation of Results

The following is intended as a guide to interpretation of ReQuest® ENA PLUS SCREENING test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

EUs	Index Value	Interpretation
<20	<0.8	Negative for anti-ENA IgG antibody.
20 - 25	0.8 – 1.0	Borderline Positive for anti-ENA IgG antibody.
> 25	>1.0	Positive for anti-ENA IgG antibody.

The suggested method for reporting results is: The following results were obtained with the ReQuest® ENA PLUS SCREENING test. A positive response is indicated by a yellow color; the calculated Enzyme Units (EU) are greater than 25 EUs. A negative response is indicated by a colorless or less intense yellow color. The calculated Enzyme Units (EU) are less than 20 EUs.

Limitations

The results obtained with the ReQuest® ENA PLUS SCREENING test serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
Confirmative testing for specific antibodies should be run if a positive assay is obtained. A positive result suggests certain diseases and should be confirmed by clinical findings.
The assay performance characteristics of ReQuest® ENA PLUS SCREENING have not been established for matrices other than serum.

Expected Values

The negative range was determined from serum samples obtained from 68 normal blood donors which were assayed by the ReQuest® ENA PLUS SCREENING. The average EU = 4.1 and Standard Deviation = 3.0. All 68 normal samples were negative for ENA antibodies.
The positive range has been stablished by ReQuest® using data obtained from 74 control sera and patient sera containing ENA antibodies. EUs ranged from a low of 62 to a high of 384 (4).

Performance Characteristics

Comparative Testing
ReQuest® ENA PLUS SCREENING test results correlated well with results of other serological tests. A side by side comparison with other commercially available ELISA ENA, Scl-70 and Jo-1 assays was done on 125 serum samples obtained from a population containing both positive and negative samples. The relative specificity and sensitivity were 96 % and 100 % respectively as compared to the reference methods (4).

Specificity
To demonstrate the specificity of the ReQuest® ENA PLUS SCREENING test, a number of ANA specific control sera containing high levels of monospecific antibodies to Sm, RNP, SS-A/Ro, SS-B/La, Jo-1, Scl-70, Centromere, ribosomal RNP, histones and dsDNA were tested using the ReQuest® ENA PLUS SCREENING. All anti-ENA sera gave positive results. All sera containing other specificities were negative.

Precision
Intra-assay precision was determined by testing a strong positive anti-ENA control and a weak positive anti-ENA control with a replication of 18 well. The CV's were 5.8 and 7.1 %.
Inter-assay precision was determined by testing a strong positive anti-ENA control and a weak positive anti-ENA control in a total of 12 assays. The CV's were 11.0 and 12.4 % (4).

References

1. White RH, Robbins DL. West J Med. 147: 210-213, 1987
2. Moore TL, et al. Seminars in Arthritis and Rheum. 10: 309-318, 1981
3. Tan EM. Advances in Immunology, 44; 83-151, 1989
4. Data on file

Manufacturer:
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L-01-330H-2
2017-06

CE

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Symbols Glossary

Symbol	Standard Title and Number	Title of Symbol	Symbol reference #	Explanatory Text
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Manufacturer	5.1.1	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Authorized representative in the European Community	5.1.2	Indicates the Authorized representative in the European Community.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Use-by-date	5.1.4	Indicates the date after which the medical device is not to be used.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Batch code	5.1.5	Indicates the manufacturer's batch code so that the batch or lot can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Catalog number	5.1.6	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Temperature limit	5.3.7	Indicates the temperature limits to which the medical device can be safely exposed.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Consult instruction for use	5.4.3	Indicates the need for the user to consult the instructions for use.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	*In vitro* diagnostic medical device	5.5.1	Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Contains sufficient for 96 tests	5.5.5	Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
Rx Only	Guidance for Industry and FDA on Alternative to Certain Prescription Device Labeling Requirements	Rx Only	N/A	Caution: Federal law prohibits dispensing without prescription.

L-01-330H-2
2017-06

CE