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User's Manual

ENA Screen ELISA

REF **DEIA-JY2433**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

ENA Screen is an ELISA-based test system for the qualitative screening of IgG class autoantibodies against extractable nuclear antigens (ENA): SS-A 60, SS-A 52, SS-B, Sm, RNP/Sm, Scl-70, and Jo-1, in human serum or plasma samples. This product is intended for professional in vitro diagnostic use only. The test is used as an aid for screening and differentiation of inflammatory autoimmune diseases, e.g. systemic lupus erythematosus, mixed connective tissue disease, Sjoegren's syndrome, scleroderma. Evaluation of a test result should always take into account all clinical and laboratory diagnostic findings.

Principles of Testing

A mixture of purified antigens SS-A 60, SS-A 52, SS-B, Sm, RNP/Sm, Scl 70 and Jo-1 is coated on to microwells. The determination is based on an indirect enzyme linked immune reaction with the following steps: Specific antibodies in the patient sample bind to the antigen coated on the surface of the reaction wells. After incubation, a washing step removes unbound and unspecifically bound serum or plasma components. Subesquently added enzyme conjugate binds to the immobilized antibodyantigen-complexes. After incubation, a second washing step removes unbound enzyme conjugate. After addition of substrate solution the bound enzyme conjugate hydrolyses the substrate forming a blue coloured product. Addition of an acid stops the reaction generating a yellow end-product. The intensity of the yellow color correlates with the concentration of the antibody-antigen-complex and can be measured photometrically at 450 nm.

Reagents And Materials Provided

1. **SORB MT** 1x divisible microplate consisting of 12 modules of 8 wells each. Ready to use.
2. **CONTROL A - C** 3x 1.5 ml Controls, containing ENA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN3 0.09%), yellow. Control A (negative), Control B (cut-off), Control C (positive). Ready to use.
3. **SAM DIL** 5x 20 ml Sample Buffer, containing PBS, BSA, detergent, preservative NaN3 0.09%, yellow, 5x conc.
4. **ENZ CONJ** 15 ml Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.
5. **SUB TMB** 15 ml TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
6. **STOP SOLN** 15 ml Stop solution; contains acid. Ready to use.
7. **WASH SOLN** 50x 20 ml Wash Buffer, containing Tris, detergent, preservative NaN3 0.09%; 50 x conc
8. **1 Instruction for Use**
9. 1 Certificate of Analysis

Materials Required But Not Supplied

1. Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
2. Data reduction software
3. Multi-channel dispenser or repeatable pipette for 100 µl

4. Vortex mixer
5. Pipettes for 10 µl, 100 µl and 1000 µl
6. Laboratory timing device
7. Distilled or deionised water
8. Measuring cylinder for 1000 ml and 100 ml
9. Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system.

Storage

1. Store test kit at 2-8°C in the dark.
2. Do not expose reagents to heat, sun, or strong light during storage and usage.
3. Store microplate sealed and desiccated in the clip bag provided.
4. Unopened reagents are stable until expiration of the kit. See labels for individual batch.
5. Diluted Wash Solution and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

Specimen Collection And Preparation

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum or plasma by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia should be avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8°C for up to five days or stored at -20°C up to six months.
5. Avoid repetitive freezing and thawing of serum or plasma samples. This may result in variable loss of antibody activity.
6. Testing of heat-inactivated sera is not recommended.

Reagent Preparation

Wash Buffer

Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised water to a final volume of 1000 ml prior to use.

Sample Buffer

Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a final volume of 100 ml.

Preparation of samples

Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well.

Note: Calibrators / Controls are ready to use and need not be diluted.

Assay Procedure

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots and products.
3. All materials must be at room temperature (20-28°C) prior to use.
4. Prepare all reagents and samples. Once started, perform the test without interruption.
5. Double determinations may be done. By this means pipetting errors may become obvious.
6. Perform the assay steps only in the order indicated.
7. Always use fresh sample dilutions.
8. Pipette all reagents and samples into the bottom of the wells.
9. To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
10. Wash microwells thoroughly and remove the last droplets of Wash Solution.
11. All incubation steps must be accurately timed.
12. Do not re-use microplate wells.

Prepare enough microplate modules for all calibrators / controls and patient samples.

1. Pipette 100 µl of calibrators, controls and prediluted patient samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28 °C).
3. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
4. Dispense 100 µl of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
7. Dispense 100 µl of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature
9. Add 100 µl of stop solution to each well of the modules
10. Incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1	A	P1								
B	B	P2	B	P2								
C	C	P3	C	P3								
D	D	P4	D	P4								
E	E	P5	E	P5								
F	F	P6	F	P6								
G	C+	P7	C+	P7								
H	C-	P8	C-	P8								
	IgG	IgG	IgA	IgA								

P1, ... patient sample A-F calibrators C+, C- controls

Quality Control

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

Calculation

For qualitative results the optical density (OD) of a sample is compared to the optical density of Control B:

Negative: OD sample < OD Control B

Positive: OD sample ≥ OD Control B

For detailed results the optical density of a sample is expressed as Index value:

Index = OD sample / OD Control B

Interpretation Of Results

Negative: Index < 1.0

Positive: Index ≥ 1.0

Performance Characteristics

Calibration

The assay system is calibrated against the internationally recognized reference sera from CDC, Atlanta USA.

Expected values

In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off Index 1.0

Sensitivity: 92.7 %

Specificity: 96.3 %

Overall agreement: 94.8 %

Study results

Study population ----- n ----- n Pos ----- %

SLE ----- 25 ----- 22 ----- 88.0

Sjogren's Syndrome ----- 10 ----- 10 ----- 100.0

MCTD ----- 10 ----- 10 ----- 100.0

Scleroderma ----- 10 ----- 9 ----- 90.0

Norman human sera ----- 60 ----- 2 ----- 3.3

RA (rheumatoid arthritis) ----- 20 ----- 1 ----- 5.0

Specificity

NA

Linearity

Patient samples containing high levels of specific antibody were serially diluted in sample buffer. Activity for each dilution step was calculated as Index- Value.

Sample	Dilution	Observed Index	Expected Index	O/E %
1	1:100	5.4	5.4	100
	1:200	2.9	2.7	107
	1:400	1.5	1.4	111
	1:800	0.7	0.7	104
	1:1600	0.4	0.3	110
2	1:100	6.3	6.3	100
	1:200	3.4	3.2	108
	1:400	1.4	1.6	91
	1:800	0.6	0.8	81
	1:1600	0.4	0.4	107

Reproducibility

Intra-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean Index	CV %
1	0.2	6.0
2	1.0	2.2
3	2.2	1.6

Inter-Assay		
Sample	Mean Index	CV %
1	0.2	6.9
2	1.1	1.2
3	2.4	1.6

Interferences

No interference has been observed with haemolytic (up to 1000 mg/dl) or lipemic (up to 3 g/dl triglycerides) sera or plasma, or bilirubin (up to 40 mg/dl) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparin). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

Precautions

1. All reagents of this kit are intended for professional use only.
2. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
3. Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
4. Avoid contact with the substrate TMB (3,3´,5,5´-Tetramethyl-benzidine).
5. Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
6. Calibrators, Controls, sample buffer and Wash buffer contain sodium azide (NaN3) 0.09% as preservative. This concentration is classified as non-hazardous.
7. Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.

During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:

8. First aid measures: In case of skin contact, immediately wash thoroughly with water and soap.

   Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
9. Personal precautions, protective equipment and emergency procedures:

   Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
10. Exposure controls / personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
11. Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
12. For disposal of laboratory waste the national or regional legislation has to be observed.

    Observe the guidelines for performing quality control in medical laboratories by assaying control sera.