

ORGENTEC Diagnostika GmbH

Carl-Zeiss-Straße 49-51

55129 Mainz - Germany

Phone: +49 (0) 61 31 / 92 58-0

Fax: +49 (0) 61 31 / 92 58-58

Internet: www.orgentec.com



**For research use only.
Not for diagnostic procedures.**

RUO 544 ENA-4-Profile

INTENDED USE

ENA-4-Profile is an ELISA test system for the qualitative measurement of IgG class autoantibodies directed against the extractable nuclear antigens SS-A (52 and 60 kDa, Ro), SS-B (La), Sm and RNP/Sm in human serum or plasma. This product is intended for research use only. Not for diagnostic procedures.



Distributed By:

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8201 Central Ave NE, Suite P

Minneapolis, MN 55432, USA

info@ibl-america.com

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SYMBOLS USED ON LABELS



Manufacturer



Catalogue number



Sufficient for 96 determinations



Batch code



Use by



Temperature limitation



Keep away from sunlight



Do not reuse



Date of manufacture



Consult instructions for use

MICROPLATE

Microplate

CALIBRATOR

Calibrator

CALIBRATOR

Calibrator

CALIBRATOR

Calibrator

CALIBRATOR

Calibrator

CONTROL +

Control positive

CONTROL -

Control negative

DILUENT

Sample Buffer P

CONJUGATE

Enzyme Conjugate

TMB

TMB Substrate

STOP

Stop solution

WASH

Wash Buffer

RTU

Ready to use

PRINCIPLE OF THE TEST

Extractable nuclear antigens SS-A (52 and 60 kDa), SS-B, Sm, RNP/Sm, are bound to microwells.

The determination is based on an indirect enzyme linked immune reaction with the following steps:

Specific antibodies in the 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. Subsequently added enzyme conjugate binds to the immobilized antibody-antigen-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.

WARNINGS AND PRECAUTIONS

- All reagents of this kit are intended for professional use only.
- 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.
- Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
- Avoid contact with the substrate TMB (3,3',5,5'-Tetramethyl-benzidine).
- Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
- Control, sample buffer and wash buffer contain sodium azide 0.09% as preservative. This concentration is classified as non-hazardous.
- 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:








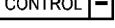
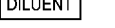
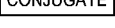
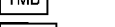
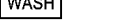


- First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into contact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
- 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.

- Exposure controls / personal protection: Wear protective gloves of nitril rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- 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.

CONTENTS OF THE KIT

RUO 544	 96	Sufficient for 96 determinations
	1	One divisible microplate consisting of 12 modules of 8 wells each. Ready to use. Color code on module
	1 ml	Calibrator SS-A (cut-off), containing SS-A antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
	1 ml	Calibrator SS-B (cut-off), containing SS-B antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
	1 ml	Calibrator Sm (cut-off), containing Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
	1 ml	Calibrator RNP/Sm (cut-off), containing RNP/Sm antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
 +	1.5 ml	Control positive, containing ENA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
 -	1.5 ml	Control negative, containing ENA antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN ₃ 0.09%), yellow. Ready to use.
	20 ml	Sample Buffer P, containing PBS, BSA, detergent, preservative sodium azide 0.09%, yellow, concentrate (5 x).
	15 ml	Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; PBS, BSA, detergent, preservative PROCLIN 0.05%, light red. Ready to use.
	15 ml	TMB Substrate; containing 3,3', 5,5'- Tetramethylbenzidin, colorless. Ready to use.
	15 ml	Stop solution; contains acid. Ready to use.
	20 ml	Wash Buffer, containing Tris, detergent, preservative sodium azide 0.09%; 50 x conc.
	1	Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised water
- Measuring cylinder for 1000 ml and 100 ml
- 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. Detailed information is provided upon request.

SPECIMEN COLLECTION, STORAGE AND HANDLING

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

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose reagents to heat, sun, or strong light during storage and usage.
- Store microplate sealed and desiccated in the clip bag provided.
- Shelf life of the unopened test kit is 18 months from day of production.

Unopened reagents are stable until expiration of the kit. See labels for individual batch.

- Diluted Wash Buffer and Sample Buffer are stable for at least 30 days when stored at 2-8°C. We recommend consumption on the same day.

PROCEDURAL NOTES

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

PREPARATION OF REAGENTS

WASH

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.

DILUENT

Sample Buffer P: 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 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.

TEST PROCEDURE

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

1. Pipette 100 µl of calibrators, controls and prediluted samples into the wells.
Incubate for 30 minutes at room temperature (20-28 °C).
Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
2. Dispense 100 µl of enzyme conjugate into each well.
Incubate for 15 minutes at room temperature.
Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
3. Dispense 100 µl of TMB substrate solution into each well.
Incubate for 15 minutes at room temperature
4. Add 100 µl of stop solution to each well of the modules
Incubate for 5 minutes at room temperature.
Read the optical density at 450 nm (reference 600-690nm) 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	C+	C+	C+	C+									Antigens coated in the modules: red = SS-A blue = SS-B yellow = Sm green = RNP
B	SS-A	SS-B	Sm	RNP									
C	C-	C-	C-	C-									
D	P1	P1	P1	P1									
E	P2	P2	P2	P2									
F	P3	P3	P3	P3									
G	P4	P4	P4	P4									
H	P5	P5	P5	P5									

red blue yellow green

P1, ... sample calibrator: SS-A, SS-B, Sm, RNP C+, C- control

VALIDATION

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 OF RESULTS

For qualitative results: for each antigen of the profile the optical density (OD) of a sample is compared to the OD of the antigen-specific Calibrator

Negative: OD sample < OD Calibrator

Positive: OD sample ≥ OD Calibrator

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

$$\text{Index} = \text{OD sample} / \text{OD Calibrator}$$

ASSAY CHARACTERISTICS

CALIBRATION

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

Measuring range

Calculation range: not applicable

Interfering substances

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, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

LIMITATIONS OF THE PROCEDURE

For research use only. Not for diagnostic procedures.

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Change Control
Former version: -

Reason for revision: *first edition*

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