

ENA-6 Profile ELISA

Enzyme immunoassay for the separate semi-quantitative detection of IgG antibodies against six nuclear and cellular antigens in human serum

REF **RE70031**

 **96**

   **2–8 °C**

EU: **IVD**  U.S.: *For research use only.*
Not for use in diagnostic procedures.



Instruction manual

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1. Intended Use

ENA-6 Profile ELISA is a solid phase enzyme immunoassay for the separate semi-quantitative detection of IgG antibodies against six cellular and nuclear antigens in human serum. The wells are coated with recombinant SS-B, SS-A 52 kDa, Scl 70, Jo-1 and highly purified native human snRNP/Sm, Sm and SS-A 60 kDa. The assay is a tool in the differential diagnosis of systemic rheumatic diseases.

2. Clinical Application and Principle of the Assay

Anti-nuclear antibodies (ANA) are an important tool for the differential diagnosis of systemic rheumatic diseases, Indirect immunofluorescence test (IFT) on eucaryotic cells like HeLa has been the established method for the detection of ANAs. Single antibody specificities are distinguished by fluorescence patterns but more specific testing by ELISAs employing the target antigens are available too for a simple and reliable differentiation of ANAs.

ANAs are especially found in active and inactive systemic lupus erythematosus (SLE), mixed connective tissue diseases (MCTD), scleroderma, Sjögren`s syndrome, polymyositis.

ANA antibodies against:

-Sm (Smith antigen) are directed against core proteins (B,B`, D1-D3, E, F, G) of small nuclear ribonucleoproteins (snRNPs). Anti-Sm as well as antibodies against double stranded DNA (dsDNA) are highly specific for SLE and thus are included in diagnostic and classification criteria for SLE.

-snRNP/Sm complex are directed against Sm and U1 snRNP proteins (70 kDa, A and C). They occur in SLE, Sjögren`s syndrome, scleroderma and polymyositis.

-SS-A (Ro; soluble cytoplasmic and/or nuclear ribonucleoproteins of 52 kDa and 60 kDa) and antibodies against SS-B (La; 48 kDa protein associated with RNA polymerase III) are mainly found in high titers for primary and secondary Sjögren`s syndrome but also in SLE, congenital heartblock and neonatal lupus.

-Scl-70 are directed against DNA-topoisomerase I. They are highly specific for systemic scleroderma and give a hint for a severe course.

-Jo-1 are directed against histidyl-tRNA synthetase (cytoplasmic protein involved in protein biosynthesis) and are found in 20-40 % of patients with polymyositis and dermatomyositis.

Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

3. Kit Contents

To be reconstituted:

5x Sample Buffer 1 vial, 20 ml - 5x concentrated (capped white: yellow solution)
Containing: Tris, NaCl, BSA, sodium azide (preservative)

50x Wash Buffer 1 vial, 20 ml - 50x concentrated (capped white: green solution)
Containing: Tris, NaCl, Tween, sodium azide (preservative)

Ready to use:

Calibrators A-D 1 vial, 1.5 ml each (capped white: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Cut-off control 1 vial, 1.5 ml (capped blue: yellow solution)
Containing: Human serum (diluted), sodium azide (preservative)

Conjugate 1 vial, 15 ml IgG (capped blue: blue solution)
Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase

TMB Substrate 1 vial, 15 ml (capped black)
Containing: Stabilized TMB/H₂O₂

Stop Solution 1 vial, 15 ml (capped white: colorless solution)
Containing: 1M Hydrochloric Acid

Microtiterplate 12x8 well strips with breakaway microwells
Coating see paragraph 1

Material required but not provided:

Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware, test tubes for dilutions. Distilled water. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or multipipette. Microplate washing device (multichannel pipette or automated system), adsorbent paper.

4. Storage and Shelf Life

Store all reagents and the microplate at 2-8°C, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 4°C, at least. ***Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.***

5. Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics shall perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Reagents may be irritating to eyes and skin thus avoid contact with eyes and skin and wear disposable gloves. Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth. All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results. Allow all components to reach room temperature (20-26°C) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test. Always pipet substrate solution with clean tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

6. Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation. Blood samples should be collected in dry tubes. After separation, the serum samples should be used immediately, respectively stored at 2-8°C up to three days, or frozen at -20°C for longer periods.

7. Assay Procedure

7.1 Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples

Dilute serum samples 1:101 with sample buffer (1x)

e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

Washing

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C).

7.2 Work flow

Pipette 100 µl of each patient's diluted serum into the designated microwells.

Pipette 100 µl of calibrators and cut-off control into the designated wells.

Incubate for 30 minutes at room temperature (20-26°C).

Wash 3x with 300 µl washing buffer (diluted 1:50).

Pipette 100 µl conjugate into each well.

Incubate for 15 minutes at room temperature (20-26°C).

Wash 3x with 300 µl washing buffer (diluted 1:50).

Pipette 100 µl TMB substrate into each well.

Incubate for 15 minutes at room temperature (20-26°C), in the dark.

Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.

Incubate 5 minutes minimum.

Agitate plate carefully for 5 sec.

Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

8. Interpretation

Establish the standard curve by plotting the **optical density (O.D.) of each calibrator (y-axis)** with respect to the corresponding concentration values in **U/ml (x-axis)**. For best results we recommend log/lin coordinates and 4-Parameter Fit. From the O.D. of each sample, read the corresponding antibody concentrations expressed in **U/ml**.

Example of interpretation

We recommend pipetting cut-off control in parallel for each run.

Calibrators/IgG	O.D. 450/620 nm
0 U/ml	0.041 OD
10 /Uml	0.480 OD
30 U/ml	0.994 OD
100 U/ml	2.094 OD
Cut-off control	
15 U/ml	0.563 OD

Normal Range
< 15 U/ml

Positive Results
> 15 U/ml

Do not use this example for interpreting patients results!

We recommend to retest samples, that are borderline. For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations.

Qualitative Calculation

Calculation of the ENA-6 Profile test can be carried out by direct comparison of the optical density (OD) of each patient sample with the optical density of the Cut-off control.

Patient samples exhibiting optical densities higher than the optical density of the Cut-off control are considered to be positive for the respective ANA-ENA autoantibodies.

9. Technical Data

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	60 minutes at room temperature (20-26°C)
Calibration range:	0-100 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C use original vials, only
Number of determinations:	96 tests

10. Performance Data

10.1 Analytical sensitivity

The analytical sensitivity of this kit has been found at 1.0 U/ml.

10.2 Specificity

The microplate is coated with highly purified and/or recombinant antigens (SS-A, SS-B, snRNP/Sm, Sm, Scl-70, Jo-1). No crossreactivities to other autoantigens have been found.

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

10.4 Calibration

The ENA-6 Profile ELISA is calibrated against reference sera from the CDC (center of disease control) Atlanta.


11. Literature

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2. **Froelich CH, Wallmann H, Skosey JL and Teodorescu M.**
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3. **Mierau R, Genth E**
Autoantikörper bei systemischem Lupus erythematodes und verwandten Erkrankungen
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TH-Books, Frankfurt, 1998, 5. Auflage: 843-851.
4. **Schmolke M, Oppermann M, Helmke K, Guder WG.**
Antibody determination against ENA- a challenge for the routine laboratory.
Poster P59, 5 th Dresden Symposium on Autoantibodies, 2000.

A: Pipetting Scheme

		Controls	Samples
Pipette	Controls	100 µl each	
Pipette	Prediluted samples (1:101)		100 µl each
Incubate	<i>30 min at room temperature (20-26°C)</i>		
Decant	<i>Wash 3x with 300 µl of wash buffer (1x)</i>		
Pipette	Conjugate	100 µl	100 µl
Incubate	<i>15 min at room temperature (20-26°C)</i>		
Decant	<i>Wash 3x with 300 µl of wash buffer (1x)</i>		
Pipette	Substrate	100 µl	100 µl
Incubate	<i>15 min at room temperature (20-26°C), in the dark.</i>		
Pipette	Stop Solution	100 µl	100 µl
Incubate	<i>5 min at room temperature (20-26°C)</i>		
<p><i>Agitate plate for 5 seconds and read OD at λ450 nm (optionally λ450/620 nm) within 30 minutes. Resulting color is stable for 30 minutes, at least.</i></p>			

Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλισμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di valutazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazemar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED. Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben. Voir MATERIEL FOURNI pour les symbôles des composants du kit. Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS. Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS. Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT. Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

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