



**REF 10103**  
**ENA-6Pro**



# Instruction Manual

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	Product Ref.	10103
	Product Desc.	ENA-6Pro
	Manual Rev. No.	005 : 2014-01-15

## 1 Intended Use

**ENA-6Pro** 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 intensity 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.

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### 3 Kit Contents

<b>TO BE RECONSTITUTED</b>				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
<b>READY TO USE</b>				
Item	Quantity	Cap color	Solution color	Description / Contents
Calibrators A-D	4 x 1.5ml	White	Yellow*	Concentration of each calibrator: 0, 10, 30, 100 Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	1 x 1.5ml	Blue	Yellow	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15ml	Blue	Blue	Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H <sub>2</sub> O <sub>2</sub> )
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.
* Color increasing with concentration				
<b>MATERIALS REQUIRED, BUT NOT PROVIDED</b>				
Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (600-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).				

### 4 Storage and Shelf Life

Store all reagents and the microplate at 2-8°C/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°F for at least 1 month. 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.

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## 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 may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of the intended use, refer to the following for maximum safety:

#### ***Recommendations and precautions***

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

**WARNING!** Calibrators, Controls and Buffers contain sodium azide (NaN<sub>3</sub>) as a preservative. NaN<sub>3</sub> may be toxic if ingested or adsorbed by skin or eyes. NaN<sub>3</sub> may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

**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 and according to national requirements.

The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

### 5.2 General directions for use

In case that the product information, including the labeling, is defective or incorrect please contact the manufacturer or the supplier of the test kit.

Do not mix or substitute Controls, Calibrators, Conjugates or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32°C/68-89.6°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

**Incubation: We recommend test performance at 30°C/86°F for automated systems.**

Never expose components to higher temperature than 37°C/ 98.6°F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

**A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic methods.**

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## 6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum samples should be used during the first 8h, respectively stored tightly closed at 2-8°C/35-46°F up to 48h, or frozen at -20°C/-4°F for longer periods

## 7 Assay Procedure

### 7.1 Preparations prior to starting

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).

To avoid mistakes we suggest to mark the cap of the different calibrators.

#### **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/35-46°F).

## 7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:



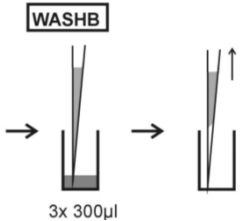
		for quantitative interpretation use calibrators to establish a standard curve						for qualitative interpretation use cut-off calibrator and CalA as negative control and CalD as positive control					
Antigen		1	2	3	4	5	6	7	8	9	10	11	12
Cal. antigen	<b>A</b>	CalA	CalB	CalC	CalD			CalA	CC	CalD			
Cal. antigen	<b>B</b>	CalA	CalB	CalC	CalD			CalA	CC	CalD			
SS-A	<b>C</b>	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
SS-B	<b>D</b>	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Sm	<b>E</b>	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
snRNP/Sm	<b>F</b>	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Scl70	<b>G</b>	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
Jo-1	<b>H</b>	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12


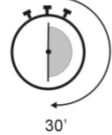
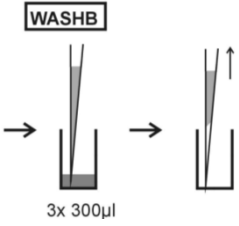

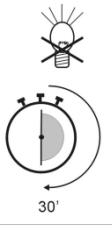
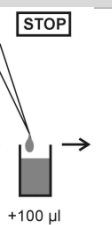

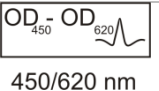
CalA: calibrator A  
CalB: calibrator B  
CalC: calibrator C

CalD: calibrator D  
CC: cut-off calibrator

P1: patient 1  
P2: patient 2  
P3: patient 3

## 7.3 Test Steps

Step	Description
1.	Ensure preparations from step 7.1 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with quantitative/ qualitative interpretation results desired:
<b>CONTROLS &amp; SAMPLES</b>	
3.	 <p>Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either:</p> <ol style="list-style-type: none"> <li>Calibrators (CAL.A to CAL.D) for <i>QUANTITATIVE</i> or</li> <li>Cut-off Calibrator (CC) for <i>QUALITATIVE</i> interp.</li> </ol> <p>and 100 µl of each of the following:</p> <ul style="list-style-type: none"> <li>Calibrator A (Cal.A) and Calibrator D (Cal.D), and</li> <li>Patients diluted serum (P1, P2...)</li> </ul>
4.	 <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p>
5.	 <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p>

CONJUGATE	
6.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Pipette 100 µl conjugate into each well.</p> </div> </div>
7.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Incubate for 30 minutes at 20-32°C/68-89.6°F.</p> </div> </div>
8.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Wash 3x with 300 µl washing buffer (diluted 1:50).</p> </div> </div>
SUBSTRATE	
9.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Pipette 100 µl TMB substrate into each well.</p> </div> </div>
10.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.</p> </div> </div>
STOP	
11.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.</p> </div> </div>
12.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Incubate 5 minutes minimum.</p> </div> </div>
13.	<p>Agitate plate carefully for 5 sec.</p>
14.	<div style="display: flex; align-items: center;"> <div style="margin-right: 20px;">  </div> <div> <p>Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.</p> </div> </div> <p>450/620 nm</p>



## 8 Qualitative and Semiquantitative Interpretation

Establish the standard curve by plotting the optical density (OD) 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 OD of each sample, read the corresponding antibody concentrations expressed in U/ml.

### Example of interpretation

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

Calibrators / IgG	OD 450/620 nm
0 U/ml	0.042 OD
10 U/ml	0.323 OD
30 U/ml	0.757 OD
100 U/ml	1.602 OD
<b>Cut-off calibrator</b>	
15 U/ml	0.451 OD

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

Patient	Replicate (OD)	Mean (OD)	Result qualitative	Result semiquantitative
P 01	0.188/0.186	0.187	negative	5.0
P 02	1.334/1.335	1.335	positive	71.4

### **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.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

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### Qualitative Calculation

Calculation of the ENA-6Pro 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 calibrator. For qualitative interpretation we recommend to consider sera within a range of 20% around the cut-off value as equivocal. All samples with higher ODs are considered positive, samples with lower ODs are considered negative.

**Negative:** OD patient < 0.8 x OD cut-off  
**Equivocal:** 0.8 x OD cut-off ≤ OD patient ≤ 1.2 x OD cut-off  
**Positive:** OD patient > 1.2 x OD cut-off

## 9 Technical Data

Sample material:	serum
Sample volume:	10 µl of sample diluted 1:101 with 1x sample buffer
Total incubation time:	90 minutes at 20-32°C/68-89.6°F
Calibration range:	0-100 U/ml
Analytical sensitivity:	1.0 U/ml
Storage:	at 2-8°C/35-46°F use original vials only.
Number of determinations:	96 tests

## 10 Performance Data

### 10.1 Analytical sensitivity

Testing sample buffer 30 times on ENA-6Pro gave an analytical sensitivity of 1.0 U/ml.

### 10.2 Specificity and sensitivity

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.

	Sensitivity
SS-A	80% for Sjögren's syndrome
SS-B	40-74% for Sjögren's syndrome
Sm	10-30% for SLE
U1-snRNP	100 % for mixed connective tissue disease
Scl 70	20-48% for systemic scleroderma
Jo-1	25% for polymyositis and dermatomyositis

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### 10.3 Linearity

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

Sample No. SCL-70	Dilution Factor	measured concentration (U/ml)	expected concentration (U/ml)	Recovery (%)
1	1 / 100	112.0	110.0	101.8
	1 / 200	56.4	55.0	102.6
	1 / 400	28.0	27.5	101.8
	1 / 800	14.3	13.8	103.6
2	1 / 100	83.8	85.0	98.6
	1 / 200	41.1	42.5	96.7
	1 / 400	20.8	21.3	97.7
	1 / 800	9.8	10.6	92.5

### 10.4 Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

Intra-assay		
ENA-6Pro	Mean OD Ratio	CV (%)
SSA	45.7	1.5
SSB	124.8	2.6
SnRNP	20.0	3.1
Sm	51.6	1.7
Scl-70	19.3	3.1
Jo-1	65.5	4.2

Inter-assay		
ENA-6Pro	Mean OD Ratio	CV (%)
SSA	44.2	1.3
SSB	123.3	2.4
SnRNP	21.7	2.8
Sm	54.6	3.9
Scl-70	22.4	3.7
Jo-1	68.4	1.7

### 10.5 Calibration

The ENA-6Pro is calibrated against reference sera from the CDC (Centers for Disease Control and Prevention) Atlanta.

## 11 Literature








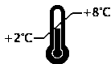












**Antinuclear antibody.** The Lancet 1984, Sept. 15: 611-13.

**Froelich CH, Wallmann H, Skosey JL and Teodorescu M.** Clinical value of an integrated ELISA system for the detection of 6 autoantibodies. The Journal of Rheumatology 1990; 17 (2): 192-200.

**Mierau R, Genth E.** Autoantikörper bei systemischem Lupus erythematodes und verwandten Erkrankungen In: Thomas L. (Hrsg.) Labor und Diagnose. TH-Books, Frankfurt, 1998, 5. Auflage: 843-851.

**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.



	- Diagnosi in vitro	- For in vitro diagnostic use
	- Pour diagnostic in vitro	- Para uso diagnóstico in vitro
	- In Vitro Diagnostikum	- In Vitro Διαγνωστικό μέσο
	- Para uso Diagnóstico in vitro	
	* Numero d'ordine	* Catalogue number
	* Référence Catalogue	* Numéro de catálogo
	* Bestellnummer	* Αριθμός παραγγελίας
	* Número de catálogo	
	* Descrizione lotto	* Lot
	* Lot	* Lote
	* Chargen Bezeichnung	* Χαρακτηρισμός παρτίδας
	* Lote	
	* Conformità europea	* EC Declaration of Conformity
	* Déclaration CE de Conformité	* Declaración CE de Conformidad
	* Europäische Konformität	* Ευρωπαϊκή συμφωνία
	* Declaração CE de Conformidade	
	* 96 determinazioni	* 96 tests
	* 96 tests	* 96 pruebas
	* 96 Bestimmungen	* 96 προσδιορισμοί
	* 96 Testes	
	* Rispettare le istruzioni per l'uso	* See instructions for use
	* Voir les instructions d'utilisation	* Ver las instrucciones de uso
	* Gebrauchsanweisung beachten	* Λάβετε υπόψη τις οδηγίες χρήσης
	* Ver as instruções de uso	
	* Da utilizzarsi entro	* Use by
	* Utilise avant le	* Utilizar antes de
	* Verwendbar bis	* Χρήση μέχρι
	* Utilizar antes de	
	* Conservare a 2-8°C	* Store at 2-8°C (35-46°F)
	* Conserver à 2-8°C	* Conservar a 2-8°C
	* Lagerung bei 2-8°C	* Φυλάσσεται στους 2-8°C
	* Conservar entre 2-8°C	
	* Prodotto da	* Manufactured by
	* Fabriqué par	* Fabricado por
	* Hergestellt von	* Κατασκευάζεται από
	* Fabricado por	
	* Calibratore cut-off	* Cut off Calibrator
	* Etalon Seuil	* Calibrador de cut-off
	* Grenzwert Kalibrator	* Οριακός ορός Αντιδραστήριο βαθμονόμησης
	* Calibrador de cut-off	
	* Controllo positivo	* Positive Control
	* Contrôle Positif	* Control Positivo
	* Positiv Kontrolle	* Θετικός ορός ελέγχου
	* Controllo positivo	
	* Controllo negativo	* Negative Control
	* Contrôle Négatif	* Control Negativo
	* Negativ Kontrolle	* Αρνητικός ορός ελέγχου
	* Controllo negativo	
	* Calibratore	* Calibrator
	* Etalon	* Calibrador
	* Kalibrator	* Αντιδραστήριο βαθμονόμησης
	* Calibrador	
	* Recupero	* Recovery
	* Corrélation	* Recuperado
	* Wiederfindung	* Ανάκτηση
	* Recuperação	
	* Coniugato	* Conjugate
	* Conjugé	* Conjugado
	* Konjugat	* Σύζευγμα
	* Conjugado	
	* Micropiastra rivestita	* Coated microtiter plate
	* Microplaque sensibilisée	* Microplaca sensibilizada
	* Beschichtete Mikrotiterplatte	* Επικαλυμμένη μικροπλάκα
	* Microplaca revestida	
	* Tampone di lavaggio	* Wash buffer
	* Tampon de Lavage	* Solución de lavado
	* Waschpuffer	* Ρυθμιστικό διάλυμα πλύσης
	* Solução de lavagem	
	* Tampone substrato	* Substrate buffer
	* Substrat	* Tampón sustrato
	* Substratpuffer	* Ρυθμιστικό διάλυμα υποστρώματος
	* Substrato	
	* Reagente bloccante	* Stop solution
	* Solution d'Arrêt	* Solución de parada
	* Stopreagenz	* Αντιδραστήριο διακοπής αντίδρασης
	* Solução de paragem	
	* Tampone campione	* Sample buffer
	* Tampon Echantillons	* Tampón Muestras
	* Probenpuffer	* Ρυθμιστικό διάλυμα δειγμάτων
	* Diluente de amostra	