

ENApró N

- 12 x 8 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG antibodies to nuclear and cytoplasmic antigens in human serum and plasma

REF	Catalogue number	LOT	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



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INTENDED USE

ENApró N is used for the semi-quantitative determination of autoantibodies to nuclear and cytoplasmic antigens in human serum or plasma.

Systemic autoimmune diseases such as systemic lupus erythematosus, scleroderma, rheumatoid arthritis, Sjögren's syndrome, dermatomyositis, mixed connective tissue disease are characterized by the appearance of a variety of autoantibodies directed against components of the cell nucleus or plasma.

Although significance and pathological relevance of some autoantibodies are not completely revealed yet, the detection of autoantibodies is widely established and plays an important role in the diagnosis of systemic autoimmune diseases (1,2,3).

ENApró N allows the detection of autoantibodies to extractable nuclear and cytoplasmic protein antigens.

ENApró N offers a rapid and handsome opportunity for the determination of the whole autoantibody pattern in systemic autoimmune diseases on one test plate. The use of specified recombinant antigens in combination with selected highly purified ones guarantees a maximum of specificity for these parameters.

- (1) Tan EM.: Antibodies to nuclear antigens (ANA) and their immunobiology and medicine. Adv Immunol 1982 33:167-240
- (2) von Mühlen CA, Tan EM: Autoantibodies in the diagnostic of systemic rheumatic diseases. Semin Arthritis Rheum 1995 24:323-358
- (3) Smeenk RJT: Antinuclear antibodies: cause of disease or caused by disease? Rheumatol 2000 39:581-584

PRINCIPLE OF THE TEST

ENApró N is an enzyme immunoassay for the semi-quantitative determination of IgG antibodies to nuclear and cytoplasmic antigens.

The antibodies of the calibrator and diluted patient samples react with nuclear and cytoplasmic antigens immobilized on the solid phase of microtiter plates. Highly purified SS-A, Sm (D), nucleosomes and histones as well as recombinant SS-B, RNP (68 kDa, A, C), Scl-70 and Jo-1 guarantee the specific binding of autoimmune antibodies of the specimen under investigation. Following an incubation period of 60 min at room temperature (RT), unbound sample components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at RT, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The cut-off is established by multiplying the OD of the calibrator with the respective factor of each antigen of the kit. Patient ratios are calculated by dividing the respective OD of the specimen with the calculated cut-off OD of the respective antigen.

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at -20 °C.

Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Note: *Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1 ml sample diluent (C), prior to assay.*

The samples may be kept at 2 - 8 °C for up to three days. Long-term storage requires -20 °C.

TEST COMPONENTS FOR 96 WELLS

A [Ag] [96]	Microtiter plate , 12 breakable strips per 8 wells (total 96 individual wells), one well per strip coated with RNP (68kDa, A, C), Sm, SS-A, SS-B, Scl-70, Jo-1, Histones and Nucleosomes respectively	1 vacuum sealed with desiccant, 2 adhesive foils
B [BUF] [WASH] [10x]	Concentrated wash buffer sufficient for 1000 ml solution	100 ml concentrate capped white
C [DIL]	Sample diluent	20 ml ready for use capped black
D [CONJ]	Conjugate containing anti-human-IgG coupled with POD	15 ml ready for use capped red
E [SOLN] [TMB]	Substrate 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use capped blue
F [H2SO4] [0.25M]	Stop solution 0.25 M sulfuric acid	15 ml ready for use capped yellow
Ca [CAL]	Calibrator (diluted serum) cut-off factors: see leaflet enclosed	2.6 ml each ready for use capped red
N [CONTROL]	Negative control (diluted serum)	2.6 ml each ready for use capped green

Materials required in addition

- micropipettes
- multi-channel pipette or multi-pipette trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- distilled or de-ionized water
- glassware

Size and storage

ENApr N has been designed for 12 x 8 determinations. This is sufficient for the analysis of 10 unknown samples as well as for the calibrator and negative control, assayed in single.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the ENApr N have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is vacuum-sealed in a foil with desiccant. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water. The wash solution prepared is stable at 2 - 8 °C up to 30 days. Crystallization of the undiluted wash buffer may occur and can be dissolved by warming up at 37 °C.

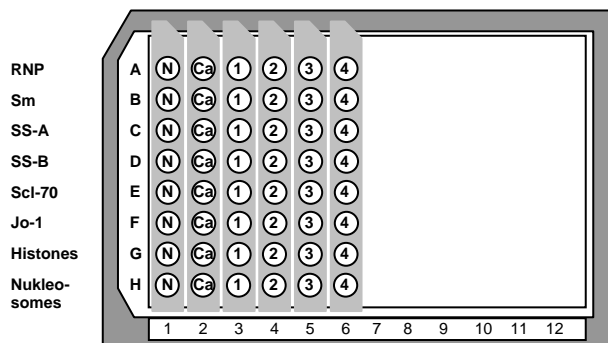
Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl sample + 1 ml sample diluent (C)
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18-25°C) before use. Mix gently without causing foam.
2. Dispense
100 µl calibrator (Ca)
100 µl negative control (N)
100 µl diluted patient samples into the respective wells.
3. Cover plate, incubate **60 min** at room temperature (18...25°C).
4. Decant, then wash each well **three times** using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Cover plate, incubate **30 min** at room temperature (18...25°C).
7. Decant, then wash each well **three times** using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Incubate **15 min protected from light** at room temperature (18...25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within 30 min after adding the stop solution.

Pipetting Format



DATA PROCESSING

Results are interpreted qualitatively by calculating a cut-off value (A) for each of the eight parameters or semi-quantitatively by calculating the binding index (BI) for each sample (B) on the basis of the cut-off determined:

(A) antigenspecific cut-off:

$$OD_{\text{calibrator}} \times \text{factor}_{\text{antigen}} = OD_{\text{cut-off antigen}}$$

The factor for each of the parameters is stated in control certificate provided in the kit. **The factor values may vary from lot to lot.**

Example:

$$OD_{\text{calibrator}} (\text{SS-A}) = 1.555$$

$$\text{factor} (\text{SS-A}) = 0.34$$

$$OD_{\text{cut-off}} (\text{SS-A}) = 1.555 \times 0.34 = 0.529$$

(B) For the calculation of the binding index (ratio) the following formula should be applied:

$$BI = OD_{\text{sample}} / OD_{\text{cut-off}}$$

Example:

$$OD_{\text{cut-off}} (\text{SS-A}) = 0.529$$

$$OD_{\text{sample}} (\text{SS-A}) = 1.859$$

$$BI = 1.859 / 0.529 = 3.5$$

This calculation can be performed by the integrated evaluation software of most microplate readers used, too.

REFERENCE VALUES

ENApr N	BI
positive	≥ 1.0
negative	< 1.0

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too. Therefore, the above mentioned reference values provide a guide only to values which might be expected.

Test validity

The test run is valid if:

- the mean OD of the calibrator is ≥ 0.7
- the mean OD of the negative control ≤ 0.3

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If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

Limitations of Method

Healthy individuals should be tested negative by the ENApr N. However, ANA/ENA positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

PERFORMANCE CHARACTERISTICS

Calibration

Due to the lack of international reference results are interpreted by calculating a BI (ratio).

Standardization

Reactivity of ANA Human Reference Sera, Center for Disease Control, Atlanta, GA, USA

CDC, Atlanta	1 h/rim	2 g	4 U1	5 Sm	7 SS-A	8 c	9 Scl70	10 Jo1
RNP	0.8	0.2	2.5	2.4	0.2	0.1	0.5	0.1
Sm	1.1	0.1	0.2	1.8	0.1	0.1	0.1	0.1
SS-A	0.1	2.9	0.2	0.1	3.3	0.3	0.4	0.1
SS-B	0.1	1.0	0.3	0.3	0.4	0.3	0.3	0.2
Scl-70	0.1	0.2	0.2	0.2	0.1	0.1	4.2	0.1
Jo-1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	4.6
Histone	1.3	0.3	0.1	0.1	0.1	0.1	0.2	0.1
Nucleo.	3.3	0.2	0.3	0.8	0.5	0.2	0.2	0.1

h homogeneous, s speckled, U1 U1-RNP, c centromere, g granular

Sensitivity

The analytical sensitivity of each reactivity of the ENApr N is around 0.2.

Intraassay variation n = 6

	BI	VK (%)
RNP	2.65	3.70
Sm	1.46	4.21
Ro/SS-A	2.54	1.90
La/SS-B	4.19	1.31
Scl-70	2.78	2.15
Jo-1	6.72	2.43
Histone	1.61	3.44
Nucleosomen	2.85	9.38

Interassay variation n = 4 x 6

	BI	VK (%)
RNP	3.36	4.55
Sm	2.31	4.50
Ro/SS-A	2.06	4.43
La/SS-B	2.34	4.22
Scl-70	2.82	4.36
Jo-1	2.57	3.44
Histone	2.84	8.95
Nucleosomen	3.16	4.17

INCUBATION SCHEME

ENAprö N (4009)

Dilute patients sample	10 µl sample + 1 ml sample diluent (C)
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1	Bring all ready for use reagents to room temperature (18...25°C) before use.				
		calibrator	control	sera	
2	Pipette	Calibrator (Ca) Negative Control (N) prediluted 1 + 100 patient sera	100 µl	100 µl	100 µl
3	Incubate	60 minutes at room temperature			
4	Wash	Decant, Dispense 3 x 300 µl (made of B)			
5	Pipette conjugate (D)	100 µl	100 µl	100 µl	
6	Incubate	30 minutes at room temperature			
7	Wash	Decant, Dispense 3 x 300 µl (made of B)			
8	Pipette substrate (E)	100 µl	100 µl	100 µl	
9	Incubate protected from light	15 minutes at room temperature			
10	Pipette stop solution (F)	100 µl	100 µl	100 µl	
11	Measure 450 nm versus 620 (690) nm				

SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- All reagents should be kept at 2 - 8 °C in the original shipping container until use.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservatives. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and for HIV as well as HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
 - Do not smoke, eat or drink while handling kit material,
 - Always use protective gloves,
 - Never pipette material by mouth,
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.