Instructions For Use

Valid as of 05.05.2025

ANApro

REF 4012

Enzyme immunoassay for the separate determination of IgG antibodies against nuclear and cytoplasmic antigens in human serum

GA Generic Assays GmbH
Ludwig-Erhard-Ring 3
15827 Blankenfelde-Mahlow OT Dahlewitz
Germany

Phone.: +49 33708 9286 0
Fax: +49 33708 4417 25

info@genericassays.com
www.genericassays.com

IVD CE Σ 12 x 8 2°C–8°C

1 Intended Purpose

The ANApro is a semi-quantitative immunoassay for the separate determination of IgG antibodies against nuclear and cytoplasmic antigens (dsDNA, RNP, Sm, SS-A, SS-B, Scl-70, CENP, Jo-1) in human serum.

The ANApro is intended as an aid in the diagnosis of systemic autoimmune disorders in conjunction with other clinical and laboratory findings.

The immunoassay is designed for manual professional *in vitro* diagnostic use.

2 Diagnostic Relevance

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.

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.

ANApro allows both the detection of autoantibodies to dsDNA as well as autoantibodies to extractable nuclear and cytoplasmic protein antigens.

ANApro 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.

3 Test Principle

The ELISA (Enzyme Linked Immunosorbent Assay) is an immunoassay for the determination of specific antibodies. The strips of the microtiter plate are coated with test-specific antigens. If antibodies are present in the patient´s sample, they bind to the antigens. A secondary antibody conjugated with the enzyme peroxidase detects the generated immune complex. A colorless substrate is converted into the colored product. The signal intensity of the reaction product is proportional to the antibody activity in the sample. After stopping the signal intensity of the reaction product is measured photometrically.

4 Test Components

Component	Description
Microtiter plate **A** **Ag | 96** 1 piece	12 breakable microtiter strips (ready-to-use), 8 wells per strip, one well per strip is coated with dsDNA, RNP (68kDa, A, C), Sm, SS-A, SS-B, Scl-70, CENP or Jo-1, respectively
Negative control **N** **CONTROL | -** 1 x 2,6 mL, green cap	Colored dilution of human serum (ready-to-use; contains ProClin 950)
Calibrator **Ca** **CAL** 1 x 2,6 mL, red cap	Colored dilution of human serum (ready-to-use; contains ProClin 950)
Sample diluent **C** **DIL** 1 x 20 mL, black cap	Colored solution (ready-to-use; contains ProClin 950)
Wash buffer **B** **BUF|WASH|10x** 1 x 100 mL, white cap	Concentrated solution (10x; contains ProClin 950)
Conjugate IgG **D** **CONJ** 1 x 15 mL, red cap	Colored solution of polyclonal anti-human IgG antibody conjugated to horseradish peroxidase (ready-to-use; contains ProClin 950)
Substrate **E** **SOLN | TMB** 1 x 15 mL, blue cap	3,3´,5,5´-Tetramethylbenzidin (ready-to-use)
Stop solution **F** **H2SO4 | 0.25M** 1 x 15 mL, yellow cap	0.25 M Sulfuric acid (ready-to-use)
Adhesive Foil 2 pieces	-
QC Certificate 1 piece	-
Instructions for Use 1 piece	-

5 Materials required but not provided

- Common laboratory equipment
- Precision pipettes (5 – 1000 µL), multi-channel pipettes (100 – 1000 µL) and disposable pipette tips
- Graduated cylinders (100 – 1000 mL)
- Sample tubes for the preparation of dilutions
- Vortex mixer or other rotators
- Microtiter plate washer or wash comb
- Microtiter plate reader with optical filters for 450 nm and 620 nm or 690 nm
- Adsorbent paper or paper towel
- Distilled or de-ionized water

6 Storage and Stability

Upon receipt, all test components must be stored at 2 °C to 8 °C, preferably in the original kit box. If stored properly in their original containers, all components are stable until their expiry date. All components are stable for at least 2 months after opening when stored properly at 2 °C to 8 °C.

7 General Information

This product is for *in vitro* diagnostic use only. The instructions for use must be carefully read before use. They are valid only for the present product with the given composition and must be strictly followed to ensure reliable test results. Deviations can lead to erroneous test results. Components must not be exchanged by test reagents of different lots or of other manufacturers.

Contamination of reagents must be avoided by use of aseptic techniques when removing aliquots from the vials. After use, reagent vials must be tightly closed with their corresponding caps.

Cross-contamination of samples or reagents can lead to inconsistent test results and must be avoided by use of consistent pipetting techniques.

Exposure of reagents to strong light must be avoided throughout the entire test procedure and storage.

Insufficient washing will result in poor precision and elevated measurement signals. After each washing step any residual fluid has to be removed completely.

8 Preparation

8.1 Preparation of Reagents

All components including the microtiter plate must be brought to room temperature (RT: 18 °C to 25 °C) before use for at least 30 min. All liquid components must be mixed gently to ensure homogeneity.

8.1.1 Microtiter Plate

The microtiter plate is sealed in an aluminium bag. Unused test strips should always be stored refrigerated and protected from moisture with the desiccant in the properly sealed aluminum bag. Carefully resealed, the test strips can be used for 8 weeks after opening.

8.1.2 Calibrators

Calibrators are ready-to-use and must not be diluted any further. Controls must be used in each test run. Laboratories can also validate their own control samples and use them alternatively.

8.1.3 Controls

The positive and negative controls are ready-to-use and must not be diluted any further. Controls must be used in each test run. Laboratories can also validate their own control samples and use them alternatively.

8.1.4 Sample Diluent

The sample diluent is ready-to-use.

8.1.5 Wash Buffer

The wash buffer is concentrated and must be diluted 1:10 with distilled water before use (e. g. 100 mL + 900 mL). A sufficient amount of washing solution must be prepared. The diluted washing solution can be stored at 2 °C to 8 °C up to 30 days.

8.1.6 Conjugate

The conjugate is ready-to-use.

8.1.7 Substrate

The substrate is ready-to-use. Exposure of the substrate solution to strong light should be avoided.

8.1.8 Stop Solution

The stop solution is ready-to-use.

8.2 Preparation of Samples

8.2.1 Sample Material

The use of freshly collected serum from blood taken by venipuncture is recommended. The use of icteric, lipemic, hemolytic or bacterially contaminated samples should be avoided. Insoluble substances must be removed from the sample by centrifugation. Samples must not be thermally inactivated.

8.2.2 Sample Dilution

The samples must be diluted 1:101 (e. g. 10 µL + 1000 µL) with sample diluent and mixed thoroughly. Building of foam should be avoided.

8.2.3 Sample Storage

Samples may be kept at 2 °C to 8 °C up to three days. Long-term storage requires -20 °C. Repeated freezing and thawing should be avoided. For multiple use, samples should be aliquoted and kept at -20 °C.

9 Test Performance

9.1 Pipetting Scheme

The following pipetting scheme is recommended:

	1	2	3	4	5
A dsDNA	N	Ca	Sample 1	Sample 2	Sample 3
B RNP	N	Ca	Sample 1	Sample 2	Sample 3
C Sm	N	Ca	Sample 1	Sample 2	Sample 3
D SS-A	N	Ca	Sample 1	Sample 2	Sample 3
E SS-B	N	Ca	Sample 1	Sample 2	Sample 3
F Scl-70	N	Ca	Sample 1	Sample 2	Sample 3
G CENP	N	Ca	Sample 1	Sample 2	Sample 3
H Jo-1	N	Ca	Sample 1	Sample 2	Sample 3

9.2 Procedure

The indicated incubation times and temperatures must be adhered to and significant time shifts during pipetting samples and reagents must be avoided. The microtiter plate should be shortly shaken after addition of reagents.

Step	Description
1. Addition of calibrators, controls and diluted samples	Add 100 µL ready-to-use calibrators and controls and diluted samples per well according to the pipetting scheme
2. Incubation	Cover the plate and incubate for 60 min. at RT
3. Wash cycle	Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets
4. Addition of conjugate	Add 100 µL ready-to-use conjugate to each well
5. Incubation	Cover the plate and incubate for 30 min. at RT
6. Wash cycle	Aspirate the solution and wash 3 times with 300 µL washing solution with at least 5 seconds soaking time each; dry by tapping the microtiter plate on a paper towel to remove any residual droplets
7. Addition of substrate	Add 100 µL ready-to-use substrate to each well
8. Incubation	Cover the plate and incubate for 15 min. in the dark at RT

9. Addition of Stop Solution	Add 100 µL ready-to-use stop solution to each well
10. Analysis	Read optical density (OD) at 450 nm versus 620 or 690 nm within 30 min. after stopping the reaction

9.3 Automation

Automated processing of the immunoassays must be performed analogous to manual use and validated by the user.

10 Test Evaluation

10.1 Metrological Traceability

The immunoassay is calibrated using reference samples from the CDC (Centers for Disease Control and Prevention, Atlanta, GA, USA). Results are expressed as Binding Index (BI).

10.2 Evaluation

The semi-quantitative evaluation is performed per antigen by calculation of the corresponding binding index (BI) by division of the optical density (OD) of the patient´s sample with the optical density of the antigen-specific cut off.

BI = OD Sample (Antigen) / OD cut off (Antigen)

The antigen-specific cut off OD is calculated according to:

OD cut off (Antigen) = OD Ca (Antigen) x Faktor (Antigen)

The antigen-specific factor for calculation of the corresponding cut off OD is indicated on the quality control certificate

10.3 Criteria of Validity

Test runs are only valid if the following criteria of validity are fulfilled:

- OD N < Ca for each antigen
- The negative control must be evaluated negative.

If these criteria are not met, the test is not valid and must be repeated.

10.4 Troubleshooting

In case of an invalid test run, the expiry dates and storage conditions, incubation times and temperatures, and precise calibration of all instruments used should be verified. If no reason for an invalid test run could be identified, please contact the supplier or manufacturer of the product.

10.5 Reference Ranges

The reference ranges are indicated below:

	Interpretation
Binding Index < 1.0	negative
Binding Index ≥ 1.0	positive

As a result of different seroprevalences in individual regions, each laboratory should verify the reference ranges by own analysis and adapt, if necessary.

10.6 Interpretation of Test Results

A positive test result indicates the presence of specific antibodies. A negative result indicates the absence of specific antibodies, but does not exclude the possibility of an autoimmune reaction. In case of a borderline test result, a reliable evaluation is not possible.

10.7 Limitations of the Method

The interpretation of test results must always be considered in combination with the clinical picture of the patient. The diagnosis should not be based on the results of a sole diagnostic method. All clinical and laboratory findings should be evaluated to state a diagnosis. For confirmation, further investigations should be carried out.

11 Performance Characteristics

11.1 Analytical Performance Characteristics

11.1.1 Precision

The precision of test results was assessed by the determination of the intra- and interassay variation by the analysis of multiple samples with different antibody activities.

	Intraassay Precision		Interassay Precision	
	BI	CV (%)	BI	CV (%)
dsDNA	1.9	6.1	2.0	6.2
RNP	2.8	5.6	3.0	10.3
Sm	4.8	6.4	3.8	10.0
SS-A	2.3	2.9	2.3	8.6
SS-B	2.3	5.6	2.3	3.0
Scl-70	2.5	2.6	2.6	9.7
CENP	3.9	3.8	4.1	7.5
Jo-1	1.8	3.5	1.9	3.0

11.2 Diagnostic Performance Characteristics

11.2.1 Diagnostic Sensitivity and Specificity

Sensitivity and specificity were assessed by the analysis of 100 samples from patients with systemic-autoimmun rheumatoid diseases and unselected blood donors.

	Diagnostic Performance
Sensitivity	> 90 %
Specificity	> 99 %

12 Warnings and Precautions

The product is designed exclusively for *in vitro* diagnostic use by qualified, authorized and trained personnel. All test components and human samples should be handled with care as potentially hazardous. Good laboratory practices (GLP) and all relevant regulations should be adhered to.

In case the product is damaged or product information including labelling is wrong or incorrect, please contact the manufacturer or supplier.

This product contains preparations of human and / or animal origin. Any material derived from human body fluids or organs used for the preparation of components were tested and found negative for HBsAg (Hepatitis B-Virus-surface Antigen) and anti-HIV as well as anti-HCV antibodies. However, all components and all patient samples should be handled as potentially hazardous in accordance with national laws and appropriate guidelines on biological safety.

As the product contains potentially hazardous materials, the following precautions should be followed: Do not smoke, eat or drink while handling kit material or samples. Avoid direct contact to kit material or samples by wearing protective gloves laboratory coat and safety glasses. Never pipette material by mouth. Wipe up spills promptly and wash the affected surface thoroughly with a decontaminant. Wash hands thoroughly after use.

Some of the reagents contain ProClin (< 1.0 %) as a preservative, may cause skin sensitization (H317) and must not be swallowed or allowed to come into contact with skin or mucosa (P280, P333+P313).

The information in the safety data sheet on possible hazards, first aid measures, measures in the event of the unintentional release of large quantities, handling and storage, personal protective equipment, information on disposal as well as information on toxicology must be observed.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent

authority of the member state in which the user and/or the patient is established.

13 Disposal

For decontamination and disposal the recommendations of the CDC as well as the relevant local and national environmental guidelines and regulations should be adhered to. Samples, potentially contaminated materials and infectious waste must be decontaminated, e.g. by autoclaving for 20 min. at 121 °C.

14 References

- Tan EM.: Antibodies to nuclear antigens (ANA) and their immuno-biology and medicine. Adv Immunol 1982 33:167-240
- von Mühlen CA, Tan EM: Autoantibodies in the diagnostic of systemic rheumatic diseases. Semin Arthritis Rheum 1995 24:323-358.
- Smeenk RJT: Antinuclear antibodies: cause of disease or caused by disease? Rheumatol 2000 39:581 – 4.

15 Symbols

	Manufacturer
	CE marking of conformity
IVD	*In vitro* diagnostic medical device
REF	Catalogue number
UDI	Unique device identifier
LOT	Batch code
	Temperature limit
	Use-by date
	Consult instructions for use
	Contains sufficient for <n> tests
	Do not re-use
	Caution
	Warning
	Biological risk
	Keep away from sunlight
Ag 96	Microtiter plate
CAL	Calibrator
CONTROL -	Negative control
DIL	Sample diluent
CONJ	Conjugate
BUF WASH 10x	Wash buffer
SOLN TMB	Substrate
H2SO4 0.25M	Stop solution

16 Changes

Changes in current Instructions for Use	
Current Version	006/05.2025
Summary of Changes	Editorial changes in chapter 4.