ANA pro (Antinuclear Antibody) ELISA

Catalog Number:
ANP31-K01

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

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v. 1.0

INTENDED USE

The Eagle Biosciences ANA pro ELISA Assay Kit is used for the separate semi-quantitative determination of autoantibodies to nuclear and cytoplasmic antigens (dsDNA, RNP, Sm, SS-A, SS-B, Scl-70, CENP, Jo-1) in human serum or plasma for the differential diagnosis of systemic rheumatic inflammatory diseases.

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 auto-antibodies are not completely revealed yet, the detection of auto-antibodies is widely established and plays an important role in the diagnosis of systemic autoimmune diseases (1,2,3).

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.

(1) Tan EM: Antibodies to nuclear antigens (ANA) and their immuno-biology 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

ANApro is an enzyme immunoassay for the separate 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 dsDNA, SS-A and Sm as well as recombinant SS-B, RNP (68 kDa, A, C), Scl-70, Jo-1 and CENP-B guarantees 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'-tetramethyl-benzidine (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 dsDNA, RNP (68kDa, A, C), Sm, SS-A, SS-B, Scl-70, CENP-B, Jo-1 respectively	1 Vacuum sealed with desiccant, 2 adhesive foils
B BUF WASH	**Concentrated wash buffer** Sufficient for 1000 ml solution 10x	100 ml concentrate capped white
C DIL	**Sample diluent**	20 ml ready for use capped black
D CONJ	**Conjugate** containing anti-human-IgG (goat), coupled with HRP	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	**Stop solution** 0,25 M sulphuric acid 0.25M	15 ml ready for use corrosive capped yellow
Ca CAL	**Calibrator** (diluted serum) cut-off factors: see leaflet enclosed	2,6 ml ready for use capped red
N CONTROL	**Negative control** (diluted serum)	2,6 ml ready for use capped green

Materials required in addition

- Adjustable micropipettes 10 - 100 µl, 100 - 1000 µl
- pipette tips
- graduated cylinders
- tubes (2 ml) for sample preparation
- distilled or de-ionized water
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm

Size and storage

ANApro 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 ANApro 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 concentra-ted wash solution 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.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

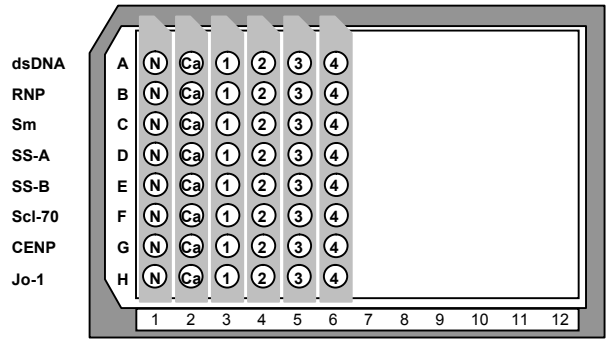
Avoid exposure of the substrate solution to light!

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) calculation of antigenspecific cut-off:

$OD_{calibrator}$ x factor $_{antigen}$ = $OD_{cut-off}$ 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:

$OD_{calibrator}$ (SS-B) = 1.900

factor (SS-B) = 0.20

$OD_{cut-off}$ (SS-B) = 1.900 x 0.20 = 0.380

(B) calculation oft he binding index (ratio) :

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

Example:

$OD_{cut-off}$ (SS-B) = 0.380

OD_{sample} (SS-B) = 1.859

BI = 1.859 / 0.380 = 4.9

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

REFERENCE VALUES

ANApro	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 ≥ 0.6
- the mean OD of the negative control ≤ 0.2

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 ANApro. However, ANA 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.

TEST CHARACTERISTICS

Calibration

Due to the lack of international reference materials 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 s	4 U1	5 Sm	7 SS-A	8 c	9 Scl70	10 Jo1
dsDNA	**2.1**	0.3	0.4	0.5	0.4	0.3	0.4	0.2
RNP	0.3	0.3	**3.2**	**4.1**	0.2	0.2	0.5	0.2
Sm	0.5	0.4	1.0	**7.5**	0.4	0.5	0.7	0.4
SS-A	0.4	**3.1**	0.6	0.4	**7.8**	0.3	1.2	0.3
SS-B	0.3	**6.1**	0.3	0.3	0.2	0.2	0.5	0.2
Scl-70	0.3	0.3	0.3	0.3	0.2	0.2	**5.0**	0.2
CENP	0.4	0.3	0.4	0.4	0.3	**5.7**	0.7	0.3
Jo-1	0.4	0.3	0.4	0.3	0.3	0.3	0.7	**9.4**

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

Sensitivity

The analytical sensitivity of each reacivity of the ANApro is around 0.2 BI.

Intra-assay variation n = 20

	BI	CV (%)
dsDNA	1.9	6.1
RNP	2.8	5.6
Sm	4.8	6.4
Ro/SS-A	2.3	2.9
La/SS-B	2.3	5.6
Scl-70	2.5	2.6
CENP	3.9	3.8
Jo-1	1.8	3.5

Inter-assay variation n = 5 x 10

	BI	CV (%)
dsDNA	2.0	6.2
RNP	3.0	10.3
Sm	3.8	10.0
Ro/SS-A	2.3	8.6
La/SS-B	2.3	3.0
Scl-70	2.6	9.7
CENP	4.1	7.5
Jo-1	1.9	3.0

ASSAY SCHEME

Dilute patient sample	**10 µl sample+ 1 ml sample diluent (C)**

1	**Bring all ready for use reagents to room temperature (18-25°C)**				
			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 (18-25°C)		
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 (18-25°C)		
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 (18-25°C)		
10	Pipette stop solution (F)		100 µl	100 µl	100 µl
11	Measure at 450 versus 620 (690) nm within 30 minutes.				

SAFETY PRECAUTIONS

- **This Eagle Biosciences ANA pro ELISA Assay Kit is for research use only**. Follow the working instructions carefully. 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 Neolone M10 (≤ 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.

Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.