

REF 10100

ANA-8S











Instruction Manual

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1 Intended Use

ANA-8S is a solid phase enzyme immunoassay for the combined qualitative detection of IgG antibodies against eight cellular and nuclear antigens in human serum. Each well is coated with recombinant 70 kDa U1-snRNP, SS-B, SS-A 52 kDa, Scl 70, centromere protein B (CenpB), 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.

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.
- U1 snRNP is directed to the 70 kDa protein of U1 snRNP. They are pathognomic for MCTD but do also occur in SLE. A high titer of antibodies against this antigen is typical for the Sharp-Syndrome.
- snRNP/Sm 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.
- CenpB (80kDa centromere protein B) are typical for the CREST-Syndrome (69% of CREST-patients), which is a more protracted type of systemic sclerosis
- 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

TO BE RECONSTITUTED					
Item	Quantity	Cap color	Solution color	Description / Contents	
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), , 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)	
	<u>'</u>	RE	ADY TO USE		
Item	Quantity	Cap color	Solution color	Description / Contents	
Negative Control	1 x 1.5ml	Green	Colorless	Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)	
Positive Control	1 x 1.5ml	Red	Yellow	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	Containing: 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 ₂ O ₂)	
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

MATERIALS REQUIRED, BUT NOT PROVIDED

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 (NaN3) as a preservative. NaN3 may be toxic if ingested or adsorbed by skin or eyes. NaN3 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).



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7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

For $*QUALITATIVE$ interpretation

	1	2	3	4
Α	NC	P2		
В	NC	P2		
С	CC	P3		
D	СС	P3		
E	PC			
F	PC			
G	P1			
Н	P1			

PC: positive control P1: patient 1

NC: negative control P2: patient 2

CC: cut-off calibrator 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 qualitative interpretation results desired:		
	CONTROLS & SAMPLES		
3.	Pipette into the designated wells as described in chapter 7.2 above, 100 μl of either: Cut-off Calibrator (CC) for <i>QUALITATIVE</i> interp.and 100 μl of each of the following: • Negative control (NC) and Positive control (PC), and • Patients diluted serum (P1, P2)		
4.	Incubate for 30 minutes at 20-32°C/68-89.6°F.		
5.	WASHB → Wash 3x with 300 µl washing buffer (diluted 1:50).		



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CONJUGATE				
6.	+100 µl	Pipette 100 μl conjugate into each well.		
7.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F.		
8.	WASHB →	Wash 3x with 300 μl washing buffer (diluted 1:50).		
		SUBSTRATE		
9.	*100 µI	Pipette 100 μl TMB substrate into each well.		
10.	30'	Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.		
		STOP		
11.	> +100 µl	Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.		
12.	5'	Incubate 5 minutes minimum.		
13.		Agitate plate carefully for 5 sec.		
14.	OD ₄₅₀ OD ₆₂₀ 450/620 nm	Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.		



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8 Qualitative Interpretation

Read the optical density of the cut-off calibrator and the patient samples. Compare patient ODs with the OD 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: $0.8 \times OD \text{ cut-off} \leq OD \text{ patient } \leq 1.2 \times OD \text{ cut-off}$

Positive: OD patient > 1.2 x OD cut-off

Calibrators	O.D. 450/620 nm	CV % (Variation)
Negative Control	0.047	2.6
Cut-off Calibrator	0.350	1.8
Positive Control	1.259	0.7

Example of interpretation.

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

Cut-off calibrator	Patient sample	OD Quotient	Interpretation
0.35 OD	0.25 OD	0.75	Negative
0.35 OD	0.40 OD	1.14	Equivocal
0.35 OD	0.56 OD	1.60	Positive
0.35 OD	1.75 OD	5.00	Positive

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 national 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.

For semi-quantification of the results, each patient-OD value can be expressed by the Index-Value. The Index-Value is calculated by dividing the patient-OD by the cut-off OD:

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Negative: Index Value < 0.8 Equivocal: 0.8≤ Index Value ≤ 1.2 Positive: Index Value >1.2

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

Storage: at 2-8°C/35-46°F use original vials only.

Number of determinations: 96 tests

10 Performance Data

10.1 Specificity and sensitivity

The microplate is coated with a mixture of highly purified and/or recombinant antigens (70 kDa U1-snRNP, SS-A, SS-B, snRNP/Sm, Sm, ScI-70, Jo-1, CenpB). No crossreactivities to other autoantigens have been found. ANA are not specific for SLE but are found in variety of rheumatic diseases. Detection of ANA is a very sensitive marker for an active SLE and is positive in >99% of all cases.

10.2 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.

Sample No.	Dilution Factor	measured concentration (OD-Ratio)	expected concentration (OD-Ratio)	Recovery (%)
1	1 / 100	3.5	3.6	97.2
	1 / 200	1.8	1.8	100.0
	1 / 400	0.9	0.9	100.0
	1 / 800	0.47	0.45	111.0
2	1 / 100	4.5	4.6	104.4
	1 / 200	2.3	2.3	100.0
	1 / 400	1.2	1.15	104.3
	1 / 800	0.6	0.58	103.5



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10.3 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			
Sample No.	Mean (OD-Ratio)	CV (%)	
1	3.7	0.8	
2	2.4	1.0	
3	1.0	1.1	

Inter-assay			
Sample No.	Mean (OD-Ratio)	CV (%)	
1	3.5	0.5	
2	2.1	0.9	
3	0.9	1.0	

10.4 Calibration

The ANA-8S is calibrated against reference sera from the CDC (Centers for Disease Control and Prevention) Atlanta.

11 Literature

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