ANA-8S IgG ELISA

For the qualitative detection of IgG antibodies against eight cellular and nuclear antigens in human serum.

Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 35-AN8HU-E01
Size: 96 wells
Version: 004: 2017-07-21 - ALPCO 2.0

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1. Intended Use

ANA-8S IgG ELISA 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.

2. Principle of the test

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Antibodies, if present in the sample, 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 sample.

3. Kit Contents

To be reconstituted:				
Item	Quantity	Cap Color	Solution Color	Description/Contents
Sample Buffer (5x)	1 x 20 mL	White	Yellow	5x concentrated Tris, sodium chloride (NaCl), sodium azide <0.1 % (preservative)
Wash Buffer (50x)	1 x 20 mL	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
Ready to Use:				
Item	Quantity	Cap Color	Solution Color	Description/Contents
Negative Control	1 x 1.5 mL	Green	Colorless	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Positive Control	1 x 1.5 mL	Red	Yellow	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Cut-off Calibrator	1 x 1.5 mL	Blue	Yellow	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, IgG	1 x 15 mL	Blue	Blue	Contains: Immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15 mL	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15 mL	White	Colorless	1 M Hydrochloric acid
Microtiter Plate	12 x 8 Well Strips	N/A	N/A	Breakaway microwell strips. Refer to first paragraph of this manual for coating description.
Materials Required, but not Provided:				

Microtiter plate reader with 450 nm reading filter and recommended 620 nm filter (600-690 nm).
Glassware (cylinder 100-1000 ml) and test tubes for dilutions.
Vortex mixer
Precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000 µl).
Microplate washing device (300 µl repeating or multichannel pipette or automated system)
Adsorbent paper.

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The test is designed to work 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 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.

5. Precautions of Use

5.1 Health hazard data

Only trained laboratory staff should perform testing with this 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 as being irritant to eyes and skin, it is recommended to avoid contact with eyes and skin and wear disposable gloves.

WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. NaN_3 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 biological 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 samples as if capable of transmitting infectious diseases and 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 ALPCO.

Do not mix or substitute reagents 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.

6. Sample Collection, Handling and Storage

The use of freshly collected serum samples is preferred. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolyzed 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.

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After separation, the serum samples should be used during the first 8 hours, respectively stored tightly closed at 2-8 °C/35-46 °F up to 48 hours (3 days), or frozen at -20°C/-4 °F for longer periods.

7. Assay Procedure

7.1 Preparations prior to pipetting

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, it is recommended to mark the cap of the different controls.

Samples:
Dilute serum samples 1:101 with 1X working strength sample buffer
e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well!

Washing:
Prepare 20 ml of 1X working 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 instrumentation and pipetting.

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, along with desiccant, seal tightly (2-8 °C/35-46 °F).

7.2 Pipetting Scheme

The following plate layout is recommended:

	1	2	3	4…
A	NC	S2		
B	NC	S2		
C	CC	S3		
D	CC	S3		
E	PC	…		
F	PC	…		
G	S1	…		
H	S1	…		

PC: Positive Control S1: Sample 1
NC: Negative Control S2: Sample 2
Cut-Off Calibrator S3: Sample 3

7.2 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:

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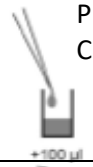
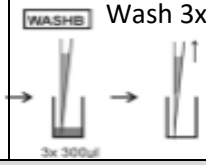
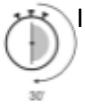
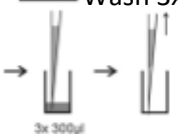
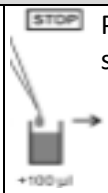
	Controls and Samples
3.	 Pipette into the designated wells as described in chapter 7.2 above, 100 µl of either: Cut-off Calibrator (CC) for QUALITATIVE interp. and 100 µl of each of the following: • Negative control (NC) and Positive control (PC), and • Sample diluted serum (P1, P2...)
4.	 Incubate for 30 minutes at 20-32°C/68-89.6°F
5.	 Wash 3x with 300 µl 1x working wash buffer.
	Conjugate
6.	 Pipette 100 µl conjugate into each well.
7.	 Incubate for 30 minutes at 20-32°C/68-89.6°F.
8.	 Wash 3x with 300 µl 1x working wash buffer.
	Substrate
9.	 Pipette 100 µl TMB substrate into each well.
10.	 Incubate for 30 minutes at 20-32°C/68-89.6°F, protected from intense light.
	Stop
11.	 Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.	 Incubate 5 minutes minimum.
13.	Shake plate carefully for 5 seconds.
14.	 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 samples. Compare sample ODs with the OD of the cut-off calibrator. For qualitative interpretation it is recommended 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: $OD_{sample} < 0.8 \times OD$ cut-off
Equivocal: $0.8 \times OD_{cut\text{-}off} \leq OD_{sample} \leq 1.2 \times OD_{cut\text{-}off}$
Positive: $OD_{sample} > 1.2 \times OD$ 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
It is recommended to pipette cut-off calibrator in parallel for each run.

Cut-off calibrator	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 sample results!

It is recommended to retest sample that are borderline. For specific data, see enclosed certificate of analysis.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment, and sample 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 must be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, plate reader, 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 ALPCO.

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

$$\text{Index Value} = \frac{\text{OD (sample)}}{\text{OD (cut-off calibrator)}}$$

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

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Storage: at 2-8°C/35-46°F use original vials, only
Number of determinations: 96 tests

10. Performance Data

10.1 Precision

Precision of test results obtained with the ANA-8S gG ELISA, were assessed by the determination of the intra- and inter assay precision as well as the lot-to-lot variance by the analysis of multiple samples of different antibody activities.

Sample ID	Intra Assay Precision		Inter Assay Precision		Lot to Lot Precision	
	Mean Value	CV	Mean Value	CV	Mean Value	CV
Sample 1	0.76	8.3 %	0.76	8.8 %	0.78	4.8 %
Sample 2	1.16	9.6 %	1.16	13.8 %	1.17	4.4 %
Sample 3	2.34	4.6 %	2.34	4.8 %	2.30	5.4%
Sample 4	4.20	2.7 %	4.20	3.1 %	4.22	3.6%
Sample 5	6.05	1.1 %	6.05	2.3 %	6.30	3.5%

10.2 Sensitivity

Sensitivity
The analytical sensitivity has been assessed by multiple analysis of sample buffer and low positive samples and calculating the limit of detection. For this kit a LoD of 0.1 (Index value) has been determined.

10.3 Calibration

This assay is calibrated against reference sera from the CDC (Centers for Disease Control and Prevention) Atlanta.

11. Disposal

Please observe the relevant statutory requirements!

12. Literature

Peter JB, Shoenfeld Y (1996). Autoantibodies. Elsevier Sciences B.V., Amsterdam.

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

Mierau R, Genth E (1998) Autoantikörper bei systemischem Lupus erythematodes und verwandten Erkrankungen In: Thomas L. (Hrsg.) Labor und Diagnose TH-Books, Frankfurt, 15. Auflage: 843-851.

Schmolke M, Oppermann M, Helmke K, Guder WG. Antibody determination against ENA-a challenge for the routine laboratory Poster P59, 5th Dresden Symposium on autoantibodies, 2000.

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CLSI Guideline GP44-A4: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

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