Version 15-09/25

User's Manual

ANA Screen 8 ELISA Kit

REF **DEIA6289**

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RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

Enzyme immunoassay for the qualitative screening of IgG antibodies against dsDNA, RNP, Sm, SS-A/Ro, SS-B/La, Scl-70, CENP-B and Jo-1 in human serum or plasma (EDTA, citrate).

General Description

Circulating autoantibodies against various intracellular antigens (antinuclear antibodies, ANA) are characteristic for systemic, autoimmune-mediated rheumatic diseases of the connective tissue (1, 2, 3, 4). These comprise Systemic Lupus Erythematosus (SLE), Mixed Connective Tissue Disease (MCTD), Sjögren's Syndrome (SS) A and B, Progressive Systemic Sclerosis (PSS, Scleroderma)/CREST Syndrome and Polymyositis (PM).

The diagnosis of the above disorders is often difficult, due to overlapping symptoms, and therefore usually supported by measuring their associated autoantibodies. 8 antigens specifically recognised by these antibodies are immobilised on the solid phase of the present enzyme-linked immunosorbent assay (ELISA): dsDNA plasmid, RNP(proteins A, C,68kDa), Sm (proteins B, B', D), SS-A/Ro(60kDa-protein), SS-B/La, Scl-70 (DNA-topoisomerase 1), CENP-B (centromere protein B), Jo-1(Histidyl-tRNA-synthetase).

The test is designed for the qualitative, summary determination of the respective autoantibodies (IgG) in human serum or plasma (cf. section 7), without the ability to discriminate between them. It is intended as initial screen test for an overall diagnosis of the above disorders. The test is fast (incubation time 30 / 30 / 30 minutes) and flexible (divisible solid phase, ready-to-use reagents). A negative and a positive control check the assay performance. The positive control also serves as calibrator for assay evaluation.

Intended Purpose

ANA Screen 8 IgG ELISA is an enzyme-linked immunosorbent assay (ELISA) intended for the qualitative, summary determination of IgG class antibodies directed against double-stranded DNA, U1-RNP complexes (proteins A, C, 68kDa), Sm, SS-A/Ro 60, SS-B/La, Scl-70 (DNA-topoisomerase 1), CENP-B (centromereprotein B) and Jo-1 (Histidyl-tRNA synthetase) in human serum or plasma samples.

Its function is the initial screening of patients with suspected systemic, inflammatory autoimmune-mediated rheumatic diseases of the connective tissue, like systemic lupus erythematosus, mixed connective tissue disease (MCTD, Sharp syndrome), Sjögren's Syndrome, Scleroderma (progressive systemic sclerosis, CREST Syndrome), polymyositis and dermatomyositis.

Principles of Testing

The wells of the solid phase are coated with a balanced mixture of the autoantigens quoted above. On this surface, the following immunological reactions take place:

1st reaction: Antigen-specific antibodies present in the sample bind to the respective immobilised antigen, forming the antigen-antibody complex. Then, non-bound sample components are washed away from the solid phase.

2nd reaction: A second antibody, directed at human IgG antibodies and conjugated with horse-radish peroxidase (HRP), is added. This conjugate binds to the complex. Then, excess conjugate is washed

away from the solid phase.

3rd reaction: The enzyme-labelled complex converts a colourless substrate into a blue product. The degree of colour development reflects the total concentration of all antigen-specific IgG autoantibodies in the sample.

Reagents And Materials Provided

1. MTP 1 Microtiter Plate, coated with a mixture of the above antigens, hermetically packed in a foil laminate pouch together with a desiccant bag. The plate consists of 12 strips, each of which can be broken into 8 individual wells.

2. SAMPLEDIL Sample Diluent, 100 mL, ready-to-use, orange coloured. Contains Tris-buffered saline (TBS), bovine serum albumin (BSA), Tween and Na-azide.

3. WASHBUF CONC Wash buffer, 100 mL, 10x-concentrate, blue coloured. Contains TBS, Tween and bromonitrodioxane.

4. CONTROL + CONTROL - Negative and Positive Control, 3.0 mL each, ready-to-use, green and red coloured, respectively. Contain TBS, BSA, Tween and Na-azide.

5. ENZCONJ IgG Enzyme Conjugate, 14 mL, ready-to-use, red coloured. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane.

6. TMB SUBS TMB Substrate Solution, 14 mL, ready-to-use, colourless. Contains a buffered solution of TMB and H_2O_2. Contained in a vial impermeable to light.

7. STOP TMB Stop Solution (0.2 M H_2SO_4), 14 mL, colourless, ready-to-use. Caution: sulfuric acid is corrosive.

8. Instructions for Use

9. Lot-specific certificate of analysis

Materials Required But Not Supplied

a. Deionised or distilled water

b. Graduated cylinder, 1000 mL

c. Tubes for sample dilution (transfer tubes in the microwell plate format recommended) d. Pipettes for 10, 100 and 1000 µL (1-and 8-channel pipettes recommended)

e. Microwell plate washer (optional)

f. Microwell plate photometer fitted with a 450 nm filter

g. ELISA evaluation program (recommended)

Storage

Store kit at 2 - 8°C, do not freeze. It is stable up to the expiry date stated on the label of the box. Do not use kit beyond its expiry date.

Reagent Preparation

Do not exchange or pool corresponding components from different kits, due to possibly different shipping or storage conditions. If the kit is to be used for several tests, only the currently needed amount of reagents should be withdrawn. It is crucially important that no cross-contamination between the reagents occurs. Use only clean pipettes and do not pour back residues into the original flasks.

1. The solid phase must reach room temperature before opening the pouch. Remove the supernumerary microwells from the frame and immediately put them back into the pouch, together with the desiccant bag. Reseal the pouch hermetically and keep it refrigerated for future use.
2. Dilute the wash buffer 10×-concentrate (100 mL, blue) with 900 mL deionised water. Mix thoroughly. The diluted buffer is stable for several weeks if stored refrigerated (2 - 8°C).
3. Preparation of the samples: handle patient specimens as potentially infectious agents. Besides serum, EDTA- or citrate-treated plasma are suitable sample material as well; heparin-treated plasma however is not.
4. Specimen requirements: highly lipemic, haemolysed or microbially contaminated samples may cause erroneous results and should be avoided.
5. Prepare samples using normal laboratory techniques. Turbid samples must first be clarified (centrifuged). The clarified or clear samples are mixed and then diluted 1/100, e.g. 10 µL serum or plasma + 990 µL sample buffer. Also mix the dilution.
6. For rapid dispensing during the assay procedure, preparation of the controls and samples in microwell transfer tubes is recommended. This allows the operation of an 8-channel pipette during the assay procedure.
7. If samples are not assayed immediately, they should be stored at 2 - 8°C and assayed within 3 days. Repeated freezing and thawing of samples should be avoided. Thawed samples must be mixed prior to diluting.

Assay Procedure

Before starting the assay, all components of the kit must have reached room temperature (23 ± 3°C).

To achieve best results, i.e. the maximum ratio between specific and background signal, careful washing is essential (steps a, c and e). It is crucially important to remove the wash solution completely. For that purpose, tap the plate firmly on several layers of absorbent tissue. Automated washers must be verified according to results obtained by manual washing.

a. Immediately prior to use, wash the solid phase once: fill wells with 350 µL wash buffer each, let soak for about 10 seconds in the wells and remove.

b. Dispense the controls (3,0 mL each, ready-to-use, green and red) and the diluted samples rapidly into the microwells; 100 µL per well. Duplicate measurements are recommended.

Incubate the plate for 30 minutes at room temperature (23 ± 3°C).

c. Wash the wells 4 times as in step a.

d. Rapidly (preferably using an 8-channel pipette) dispense the conjugate (14 mL, ready-to-use, red); 100 µL per well. Incubate the plate as in step b.

e. Repeat wash step c.

f. Rapidly (preferably using an 8-channel pipette) dispense the substrate solution (14 mL, ready-to-use,

colourless, black vial); 100 µL per well. Incubate the plate as in step b. As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) during incubation.

g. Rapidly (preferably using an 8-channel pipette) dispense the stop solution (14 mL, ready-to-use, colourless. Caution: corrosive!); 100 µL per well. Use the same sequence as for the substrate. The colour changes from blue to yellow. Agitate the plate, preferably on an orbital shaker, for about 10 seconds.

h. Immediately read the absorbance in the microwell plate photometer at 450 nm.

Refrigerate the remainder of the reagents (2 - 8°C) if they are to be used again.

Quality Control

The assay is evaluated in a qualitative manner: the absorbance of the samples is compared to the borderline absorbance (= cut-off absorbance). The cut-off absorbance is determined by means of the positive control which at the same time functions as calibrator; according to the formula:

$absorbance_{borderline}$ = absorbancepositive control × factor

The factor depends on the kit lot and is quoted in the lot-specific certificate of analysis (included with each test kit). Example:

$absorbance_{positive\ control}$ = 1250 mOD

factor = 0.35

$absorbance_{borderline}$ = 1250 mOD × 0.35 = 438 mOD

In order to gain an impression of the degree of a sample's reactivity, the ratio between sample and borderline absorbance is calculated:

ratio = $absorbance_{sample}$ / $absorbance_{borderline}$

Example:

$absorbance_{borderline}$ = 438 mOD

$absorbance_{sample}$ = 1480 mOD

ratio = 1480 mOD / 438 mOD = 3.4

Quality control: the positive control (calibrator) and negative control check the assay performance. Their acceptable ranges are quoted in the lot-specific certificate of analysis. Values of the controls must fall within the indicated ranges; otherwise, the results of the assay are invalidated.

Interpretation Of Results

Based on the measurement of a blood donor and a positive collective of sera (see below), we suggest for the assessment of patient sera:

	ratio
normal (negative) range	< 0,82
cut-off	1,00
equivocal range	0,82 - 1,20
positive range	> 1,20

These specifications are given as an indication only; in order to check their accuracy, each analysis should include parallel samples of normal sera.

A negative test result indicates that the patient probably does not have an elevated level of IgG antibodies to the antigens listed in the beginning. Therefore, presence of a systemic rheumatic disease is rather unlikely but can nevertheless not be excluded.

A positive result should be considered as an indication for one of the above listed diseases. As follow-up diagnosis, the specificity of the causative autoantibody and hence the identity of the autoimmune disorder should be determined. This can be achieved by means of a differentiating profile ELISA.

Specimens exhibiting results within the borderline range quoted above should be considered as equivocal and reported as such. It is recommended that a second sample be collected two weeks later and run in parallel with the first sample to document a possible change of antibody titer.

As with any serological test, the results should be interpreted in the light of the patient's symptoms and other diagnostic criteria.

Standardisation

The test is standardised with a purified serum preparation containing IgG antibodies directed at each of the immobilised autoantigens. It constitutes the stock material for both controls of the test. The proportion of the antibodies is adjusted in such a manner that each one contributes approximately the same fraction to the overall signal.

The stock preparation is calibrated against a set of monospecifically positive sera solely reserved for this purpose. The degree of sample reactivity is expressed as summary ratio, as outlined above.

Reference Values

Frequency distribution of ANAs (IgG):

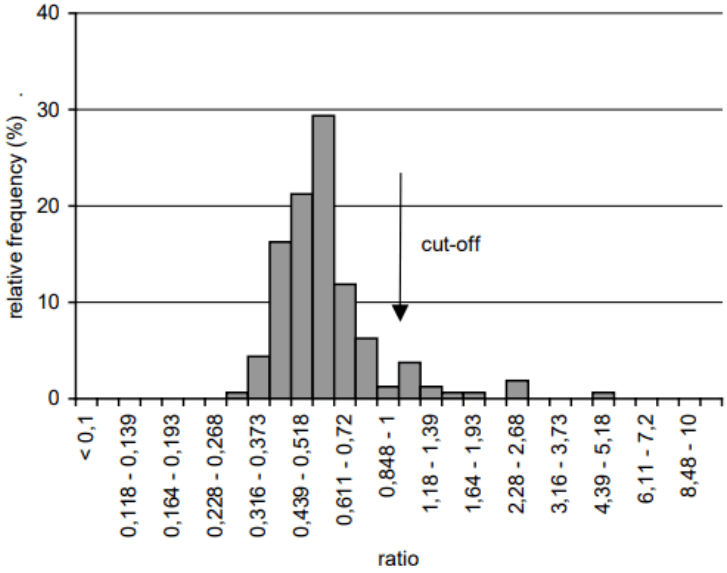
This was analysed in a sera collective of blood donors, equally distributed by sex and age, and a collective of sera which had been found positive by independent methods (e.g. monospecific, CE-compliant reference ELISAs, immune fluorescence assays (IFA)) for at least one parameter or were clinically defined.

The following summary distribution of the analytes was observed (s = standard deviation):

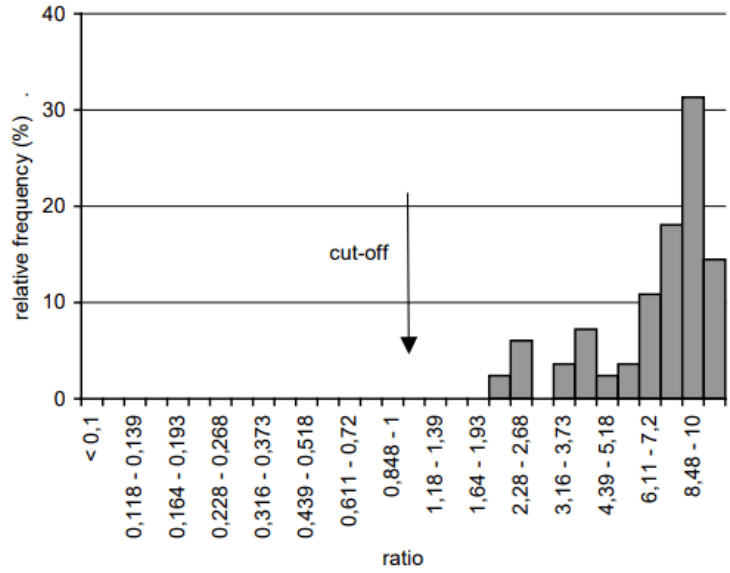
blood donor sera		positive sera	
n:	160	n:	83
mean:	ratio = 0,64	mean:	ratio = 7,5
mean + s:	ratio = 1,08	mean - s:	ratio = 4,9
mean + 2s:	ratio = 1,53	mean - 2s:	ratio = 2,4
median:	ratio = 0,54	median:	ratio = 8,2
95th percentile:	ratio = 1,14	5th percentile:	ratio = 2,5

ROC-analysis of these data was used to determine the cut-off of the ANA Screen 8 IgG ELISA. The data presented here suggest a diagnostic specificity and sensitivity of the ELISA of about 91 and nearly 100 %, respectively. These values apply for the measured sera only; other collectives may yield different results.

blood donor sera



positive sera



Precision

For the assessment of the test precision, the variability of results under the following conditions was determined: a. within 1 assay and between 3 assays, b. between 3 operators and c. between 2 kit lots.

a. Intra- and inter-assay variability (n = 24 and 72, respectively)

sample	ratio	variability (cv, %)	
		intra-assay	inter-assay
1	1,4	1,9	2,1
2	2,4	2,0	2,1
3	4,0	1,4	1,7

b. Operator to operator variability (n = 12)

sample	ratio	variability (cv, %)
1	1,4	2,5
2	2,4	1,8
3	4,0	2,9

c. Variability between 2 kit lots (n = 6)

sample	ratio	variability (cv, %)
1	1,3	4,9
2	2,3	5,3
3	3,8	3,8

Detection Limit

The detection limit is defined as that concentration of analyte that corresponds to the mean absorbance of sample buffer plus 3-fold standard deviation (s). It was determined as < 0,3 (ratio; n = 24).

Recommended measuring range: 0,4 < ratio < 6

Specificity

The test permits the specific determination of human IgG antibodies, directed at the autoantigens quoted in article 1. It has been validated (among other criteria) using human reference sera which are commercially available. The following results (ratio values) are typical:

Serum	1	2	3	4	5	6	7	8	9	10
CDC-result	ds-DNA	SS-B /La	--	U1-RNP	Sm	--	SS-A /Ro	--	Scl-70	Jo-1
Immun-fluoresc.	homo-gen	speck-led	speck-led	--	--	nuc-leolar	--	centro-mere	--	--
ratio	5,0	11	9,4	3,8	9,2	1,2	6,6	5,9	6,6	9,4

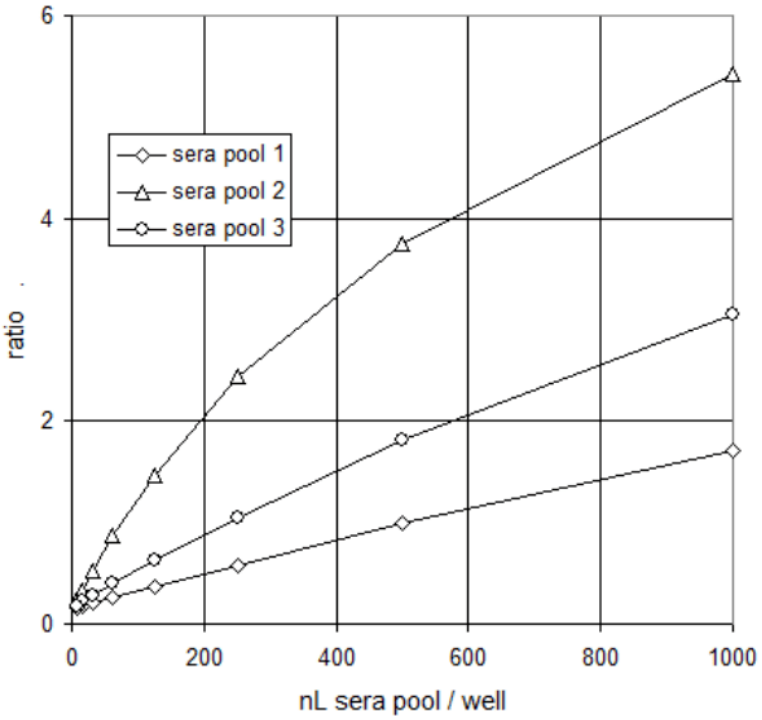
Remark: The corresponding ANA Profile 8 IgG ELISA which differentiates between the antigens revealed that serum # 6 shows ratio values ≤ 1 towards all single antigens.

Interference with anticoagulants (EDTA, Citrat, Heparin) in samples has been tested and no interference effects have been observed.

Linearity

Dose-response relationship

In order to assess this feature of the ELISA, several pools of individual sera with heterogeneous reactivity were measured in serial 2-fold dilution. A typical result is depicted below. An approximately linear relationship between sample concentration and resulting ratio is restricted to ratio values < 2. This is due to the qualitative evaluation manner (cf. Quality Control) and contrasts ELISAs which are evaluated quantitatively by means of a standard curve.



Precautions

It must be executed by trained professional staff.

The kit has been tested for transport stability and can be shipped unrefrigerated for up to 3 days. Store at 2 -

8°C on arrival. In case of doubt, contact your local distributor or the manufacturer.

Do not use reagents beyond their expiration dates. Adherence to the protocol is strongly recommended.

The sample buffer and controls contain Na-azide as antimicrobial agent. The wash buffer contains bromonitrodioxane and the conjugate methylisothiazolone / bromonitrodioxane as preservative. The substrate contains 3, 3', 5, 5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2). The stop solution, 0,2 M sulfuric acid (H_2SO_4), is acidic and corrosive.

The above mentioned reagents may be toxic if ingested. Follow routine precautions for handling hazardous chemicals. Avoid all body contact, wear gloves and eye protection. If one of the reagents comes into contact with skin or mucous membrane, wash thoroughly with water. Never pipette by mouth. Dispose in a manner complying with local/national regulations.

Na-Azide may react with lead and copper plumbing to form explosive metal azides. On disposal, flush with a large amount of water to prevent azide build-up.

The controls contain components of human origin. They were tested for human immunodeficiency virus (HIV)-Ag, hepatitis B surface (HBs)-Ag and antibodies against HIV 1/2 and hepatitis C virus (HCV) and showed negative results; either in an FDA-approved or a CE-compliant test, according to European Directive 98/79/EC.

However, no test can guarantee that material of human origin is not actually infectious. The preparations should therefore be treated as potentially infectious and disposed of accordingly, as should the samples (and residues thereof); according to CDC (Center of Diseae Control, Atlanta, USA) or other local / national guidelines on laboratory safety and decontamination.