Version 18-02/26

User's Manual

ANA Screen 8 ELISA

REF **DEIA1677**

Σ **96T**

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

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

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

The test is designed for the qualitative, summary determination of the respective autoantibodies (IgG) in human serum or plasma, without the ability to discriminate between them. 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.

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.

This product is intended for manual professional research use only.

Not for use in diagnostic procedures.

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:

1. 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.
2. 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.
3. 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 H2O2. 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 10x-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 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 1, 2 and 5). 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.

1. 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.
2. 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).
3. Wash the wells 4 times as in step 1.
4. 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 2.
5. Repeat wash step 3.
6. 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 2. As the substrate is photosensitive, avoid intense light exposure (e.g. direct sunlight) during incubation.
7. 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.
8. 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.

SUMMARY FLOW CHART

a. Dilute the samples 1/100 in sample buffer (100 mL, ready-to-use, orange) and mix.

b. Dilute the wash buffer 10x-concentrate (100 mL, blue) with water and mix.

c. Wash the wells once with 350 µL wash buffer each. Dispense 100 µL of the controls (3,0 mL each, ready-to-use, green and red) and of the diluted samples into the wells of the solid phase. Duplicate measurements are recommended. Incubate for 30 minutes at room temperature (23 ± 3°C).

d. Wash the wells 4 times with 350 µL wash buffer each.

e. Dispense 100 µL of the conjugate (14 mL, ready-to-use, red) into the wells. Incubate as in step c.

f. Repeat washing step d.

g. Dispense 100 µL of the substrate solution (14 mL, ready-to-use, black vial) per well. Incubate as in step c. Then, add 100 µL stop solution (14 mL, ready-to-use, colourless) per well and agitate the plate briefly.

h. Immediately measure the absorbance at 450 nm.

i. Evaluation: determine the borderline absorbance by multiplying the absorbance of the positive control with the factor quoted in the certificate of analysis. Then, calculate the ratio of the samples by dividing their absorbance by the borderline absorbance.

Calculation

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:

$$absorbance_{borderline} = absorbance_{positive\ control} \times factor$$

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.

Performance Characteristics

1. 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.

2. Analytical specificity

The test permits the specific determination of human IgG antibodies, directed at the autoantigens quoted in article 1. 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.

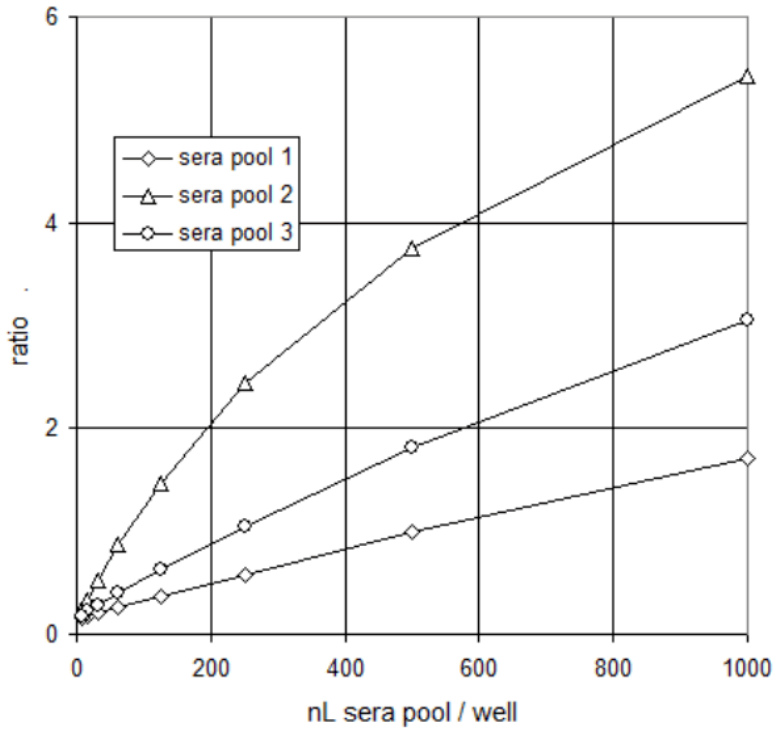
3. Detection limit (analytical sensitivity)

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

4. 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 and contrasts ELISAs which are evaluated quantitatively by means of a standard curve.



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

Precautions

For research Use only. Not for use in diagnostic procedures. Not for internal or external use in humans or animals. 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.