DRG® ANA Profile-8 ELISA (EIA-4325)

CE

REVISED 1 JUNE 2011 RM (VERS. 6.1) **RUO IN THE USA**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

The test is designed for determination of IgG autoantibodies in human serum, directed against one of the above antigens. The test is fast (incubation time 30 / 30 / 30 minutes) and flexible (divisible solid phase for 1 - 12 analyses, ready-to-use reagents).

WARNINGS AND PRECAUTIONS

The test kit is not for internal or external use in humans or animals.
Do not use reagents beyond their expiration dates. Adherence to the protocol is strongly recommended.
The sample buffer and the controls contain Na-azide as preservative. 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, 0,5 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, 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 have produced negative results when tested for Human Immunodeficiency Virus (HIV)-Ag, hepatitis B surface (HBs)-Ag, HIV 1/2-Ab and hepatitis C Virus (HCV)-Ab, in FDA-approved or European Directive 98/79/EG-compliant tests. However, no known test can guarantee that products derived from human blood will not be infectious. They should therefore be handled as if capable of transmitting infectious agents, and discarded appropriately. Please refer to CDC (Center of Disease Control, Atlanta, USA) or other local/national guidelines on laboratory safety and decontamination procedures.

PRINCIPLE OF THE TEST

The wells of the solid phase are coated with the 8 autoantigens quoted above, line by line. On this surface, the following immunological reactions take place:

1st reaction: Antigen-specific antibodies present in the sample bind to the respective immobilized 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 and 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 in each line of the solid phase reflects the concentration of the respective antigen-specific autoantibody (IgG) in the sample (8 values per sample).

CONTENTS OF THE KIT

a. 1 **microwell plate**, coated line by line with 8 individual autoantigens, as described above. Hermetically packed in a foil laminate pouch together with a desiccant bag. The plate consists of 12 strips, thus providing maximum flexibility and economy in use of the assay.

MWP	12x8

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

BUF	SPL

c. **Wash buffer**, 100 mL, 10x-concentrate, blue coloured. Contains TBS, Tween and bromonitrodioxane.

BUF	WASH	10x

d. **Negative and positive control**, 3,0 mL each, ready-to-use, green and red coloured, respectively. Contain TBS, BSA, Tween and Na-azide.

CONTROL	–

CONTROL	+

e. **Anti-human IgG HRP conjugate**, 14 mL, ready-to-use, red coloured. Buffered solution containing stabilising protein, methylisothiazolone and bromonitrodioxane.

CONJ	IgG

f. **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.

SUBS	TMB

g. **Stop solution** (0,5 M H_2SO_4), 14 mL, colourless, ready-to-use. Caution: sulfuric acid is corrosive.

SOLN	STOP

h. Directions for use

i. Lot-specific certificate of analysis

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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 OF THE KIT

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

REAGENT AND SAMPLE PREPARATION / SPECIMEN REQUIREMENTS

Do not exchange or pool corresponding components from different kits, due to possibly different shipping or storage conditions.

a. Before opening the pouch of the solid phase, it must have reached room temperature. Remove supernumerary microwell strips 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.

b. 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).

c. Preparation of the samples:
Handle specimens as if capable of transmitting infectious agents.
Prepare sera using normal laboratory techniques and dilute them **1/100**, e.g. 10 µL serum + 990 µL sample buffer. Mix thoroughly.
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.
If samples are not assayed immediately, they should be stored at 2 - 8°C and assayed within 3 days.
For longer storage, -20°C or lower temperature are recommended.
Repeated freezing and thawing of sera should be avoided. Thawed samples must be mixed prior to diluting.
Specimen requirements: Highly lipemic, hemolyzed or microbially contaminated sera may cause erroneous results and should be avoided.

ASSAY PROCEDURE

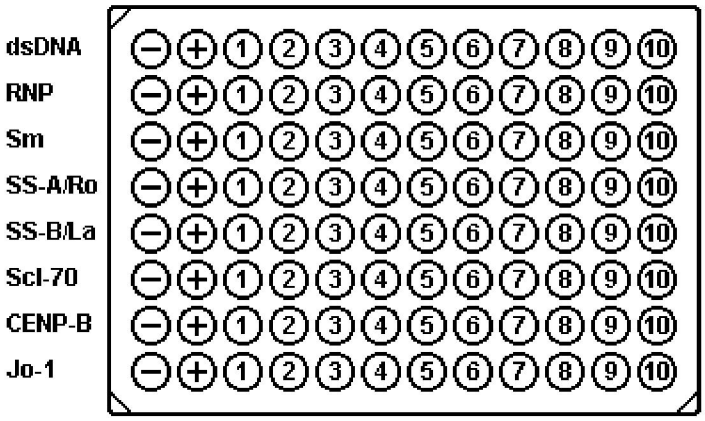
a. Manual operation

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, 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 (1 - 10) rapidly into the microwells, as depicted below; 100 µL per well.



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.

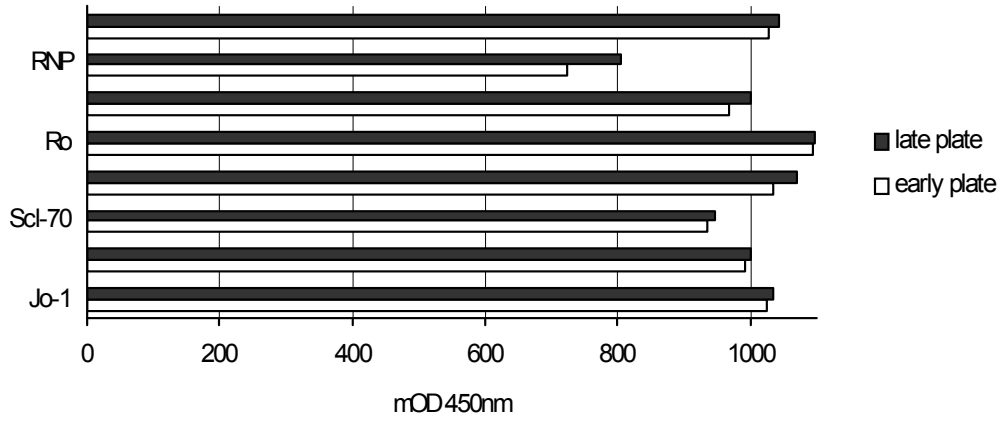
g. Rapidly (preferably using an 8-channel pipette) dispense the stop solution (14 mL, ready-to-use, colourless. Caution: corrosive!); 100 µL per well. Use the same sequence as for the substrate. The colour changes from blue to yellow. Agitate the plate, preferably on an orbital shaker, for about 10 seconds.

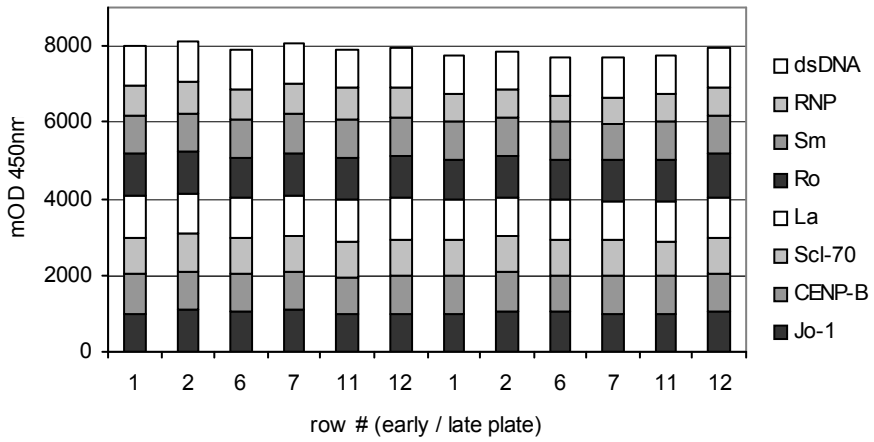
h. Immediately read the absorbance in the microwell plate photometer at 450 nm.

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

b. Dynex DS2 automated ELISA system

This product has been validated for use with the Dynex DS2 automated ELISA system. A suitable program file for assay execution and evaluation is available on request. The parameters of this program are merely a proposal and may need to be adapted by the operator to the requirements of the actual assay. In general terms, we have attempted to stick as close as possible to the protocol of manual operation, as above. However, due to the necessarily elevated temperature within the DS2, the substrate incubation period had to be shortened. Article 11.8. gives a performance comparison between manual assay operation and the DS2 ELISA system.



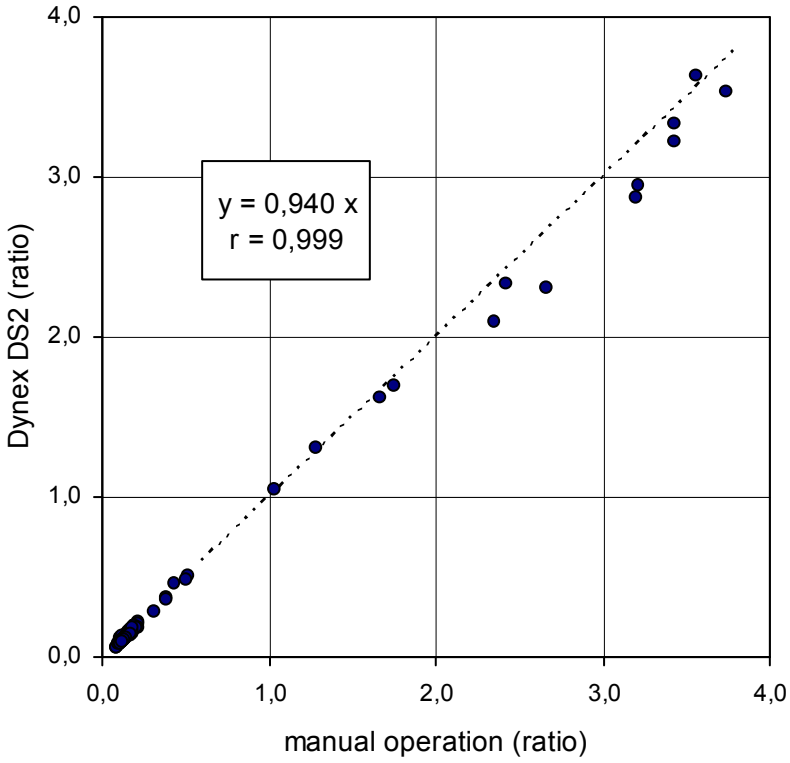


c. Manual operation vs. Dynex DS2 automated ELISA system

Variability:
Using specimen of one and the same kit lot, the variability of assay results were compared between manual operation and the Dynex DS2 automated ELISA system (cv values are given as mean of all 8 antigens):

	manual operation	Dynex DS2
intra-assay variability (n per parameter = 3)	mean cv = 1,4 %	mean cv = 1,3 %
inter-assay variability (n per parameter = 9)	mean cv = 1,6 %	mean cv = 2,0 %

Correlation:
In order to assess this feature of the ANA Profile 8 ELISA, 10 appropriately diluted CDC sera (cf. article 11.2) were measured on all 8 antigens with both methods. This is the result.



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WARRANTY

DRG guarantees that the product delivered has been thoroughly tested to ensure that its properties specified herein are fulfilled. No further warranties are given.
The performance data presented here were obtained using the procedure indicated. Any modification in the procedure may affect the results in which case DRG disclaims all warranties whether expressed, implied or statutory. Moreover, DRG accepts no liability for any damage, whether direct, indirect or consequential, which results from inappropriate use or storage of the product.

REFERENCES / LITERATURE

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SUMMARY FLOW CHART

a. Dilute the sera 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 8 ∗ 100 µL of the controls (3,0 mL, ready-to-use, green and red) and of the diluted samples into the wells of 1 column each. 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: For each parameter, determine the borderline absorbance by multiplying the respective absorbance of the positive control with the corresponding factor quoted in the certificate of analysis. Then, calculate the ratios of each sample by dividing its respective absorbance values by the corresponding borderline absorbance.

Version 2011-04-12~rm