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User's Manual

Rheumatoid Factor IgA/IgG/IgM ELISA

REF **DEIA1808**

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.

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PRODUCT INFORMATION

Intended Use

Rheumatoid Factor IgA/IgG/IgM ELISA is a solid phase enzyme immunoassay with highly purified Fc fragment of human immunoglobuline (IgG) for the separate quantitative and qualitative detection of IgG, IgM and IgA rheumatoid factors (RF) in human serum.

General Description

Rheumatoid factors (RF), first described in 1940 as antibodies reacting with gamma globulins, are autoantibodies directed against the C-terminal part of the constant region of the IgG heavy chain, the IgG Fc.

Although named after the disease they were initially associated with, RFs are found both in the healthy population and several diseases. The diseases commonly associated with high RF concentrations are rheumatoid arthritis (RA; 50-90%) and Sjögren`s syndrome (75-95%).

They are also found in systemic lupus erythematosus (SLE; 15-35%), systemic sclerosis (20-30%) polymyositis / dermatomyosisits (5-10%), cryoglobulinemia (40-100%) and mixed connective tissue diseases (MCTD; 50-60%).

Although the presence of IgM RF in the serum has been regarded as the most important serological indicator for RA, thus included in the ACR list of criteria for the diagnosis of this disease, RF of IgG and IgA subclass are important for diagnosis as well.

Determination of these isotypes provides additional information with regard to diagnosis, differential diagnosis and follow-up of RA in comparison to conventional techniques such as latex agglutination test and nephelometry. Whilst RF of subclass IgM are most sensitive for diagnosis of RA, thus most suitable for screening, RF of subclass IgG are most specific for RA and like subclass IgA correlate with clinical parameters and disease activity. The presence of all three subclasses together is 100 % specific for RA.

RF in SLE are associated with sicca syndrome, hypergammaglobulinemia, high titer of antinuclear antibodies, anemia and usually SS-A and SS-B antibodies appearance. All three subclasses are found in SLE. Especially the subclass IgA defines a subgroup of SLE patients characterized by distinct autoimmune phenomena and high disease activity in the absence of nephritis.

Principles of Testing

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.

Reagents And Materials Provided

1. Sample Buffer (5×): 1 × 20 mL. 5 × concentrated. Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).

2. Wash Buffer (50×): 1 × 20 mL. 50 × concentrated. Tris, NaCl, Tween 20, sodium azide < 0.1%(preservative)

3. Negative Control: 1 × 1.5 mL. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

4. Positive Control: 1 × 1.5 mL. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

5. Cut-off Calibrator: 1 × 1.5 mL. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)

6. Calibrators: 6 × 1.5 mL. Concentration of each calibrator: 0, 3, 10, 30, 100, 300 U/ml. Human serum (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative).

7. Conjugate, IgG: 1 × 15 mL.

Conjugate, IgM: 1 × 15 mL.

Conjugate, IgA: 1 × 15 mL.

Containing: Anti-human immunoglobulins conjugated to horseradish peroxidase, bovine serum albumin (BSA)

8. TMB Substrate: 1 × 15 mL.

9. Stop Solution: 1 × 15 mL. 1M Hydrochloric Acid

10. Microtiter plate: 12 × 8 well strips. With breakaway microwells. Refer to paragraph 1 for coating.

Materials Required But Not Supplied

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.).

Storage

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.

Specimen Collection And Preparation

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.

Reagent Preparation

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.

a. Samples:

Dilute serum samples 1:101 with sample buffer (1×)

e.g. 1000 µl sample buffer (1×) + 10 µl serum. Mix well !

b. Washing:

Prepare 20 ml of diluted wash buffer (1×) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

c. Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

d. 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.

e. 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).

2. Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

NOTE: If IgG, IgA and IgM are determined in parallel, calibrators, controls and samples have to be done for each subclass separately.

For *QUANTITATIVE* interpretation

	1	2	3	4...
A	Cal A	Cal E	P1	
B	Cal A	Cal E	P1	
C	Cal B	Cal F	P2	
D	Cal B	Cal F	P2	
E	Cal C	PC	P3	
F	Cal C	PC	P3	
G	Cal D	NC	...	
H	Cal D	NC	...	

For *QUALITATIVE* interpretation

	1	2	3	4...
A	NC	P2		
B	NC	P2		
C	CC	P3		
D	CC	P3		
E	PC	...		
F	PC	...		
G	P1	...		
H	P1	...		

CalA: calibrator A
CalB: calibrator B
CalC: calibrator C
CalD: calibrator D
CalE: calibrator E
CalF: calibrator F
PC: positive control
NC: negative control
CC: cut-off calibrator
P1: patient 1
P2: patient 2
P3: patient 3

Assay Procedure

1. Ensure preparations from step **Preparations prior to starting** above have been carried out prior to pipetting.
2. Use the following steps in accordance with quantitative/ qualitative interpretation results desired:
3. Pipette into the designated wells as described in **Pipetting Scheme** above, 100 µl of either:
   a. Calibrators (CAL.A to CAL.F) for QUANTITATIVE or
   b. Cut-off Calibrator (CC) for QUALITATIVE 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. Wash 3× with 300 µl washing buffer (diluted 1:50).
6. Pipette 100 µl conjugate into each well.
7. Incubate for 30 minutes at 20-32°C/68-89.6°F.
8. Wash 3× with 300 µl washing buffer (diluted 1:50).
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.
11. Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12. Incubate 5 minutes minimum.
13. Agitate plate carefully for 5 sec.
14. Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

Interpretation Of Results

Quantitative and Qualitative Interpretation

For quantitative interpretation establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in U/ml (x-axis). For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding

antibody concentrations expressed in U/ml.

Normal Range	Equivocal Range	Positive Results
< 12 U/ml	12 - 18 U/ml	>18 U/ml

Example of a standard curve

Do NOT use this example for interpreting patient's result

Calibrators IgG/A/M	OD 450/620 nm	CV % (Variation)
0 U/ml	0.035	2.3
3 U/ml	0.138	2.6
10 U/ml	0.342	3.2
30 U/ml	0.632	3.2
100 U/ml	1.216	0.5
300 U/ml	2.178	0.1

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	0.872/0.922	0.897	54.7
P 02	1.159/1.188	1.174	86.3

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.
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 qualitative interpretation read the optical density of the cut-off calibrator and the patient samples. Compare patient´s OD 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 × OD cut-off
Equivocal: 0.8 × OD cut-off ≤ OD patient ≤ 1.2 × OD cut-off
Positive: OD patient > 1.2 × OD cut-off

Performance Characteristics

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

Calibration range: 0-300 U/ml

Analytical sensitivity: 1.0 U/ml

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

Calibration: Due to the lack of international reference calibration this assay is calibrated in arbitrary units (U/ml) for IgG and IgA rheumatoid factors. For IgM rheumatoid factor, the assay is calibrated against the international WHO standard and results are given in IU/ml.

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 (U/ml)	CV (%)
1	15.2	0.4
2	43.4	4.5
3	288.8	8.9

Inter-assay		
Sample No.	Mean (U/ml)	CV (%)
1	18.3	1.0
2	52.1	4.6
3	322.7	8.2

Sensitivity

Analytical sensitivity: Testing sample buffer 30 times on Rheumatoid Factor IgA/IgG/IgM ELISA gave an analytical sensivity of 1.0 U/ml.

Specificity and sensitivity: The microplate is coated with Fc fragment of human immunoglobuline (IgG). No crossreactivities to other autoantigens have been found. Rheumatoid factors are detected in 70-90% of patients with rheumatoid arthritis (RA).

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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 (U/ml)	Expected (U/ml)	Recovery (%)
1	1 / 100	51.1	53.4	95.7
	1 / 200	25.2	26.7	94.4
	1 / 400	12.4	13.4	92.5
	1 / 800	6.3	6.7	94.0
2	1 / 100	135.1	138.0	97.9
	1 / 200	74.0	69.0	107.2
	1 / 400	32.1	34.5	93.0
	1 / 800	16.1	17.3	93.0

Precautions

1. Health hazard data

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 (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 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.

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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