Version 26-05/24

User's Manual

Anti-Nucleosomes Antibody ELISA Kit

REF **DEIA-JY2407**

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

The kit is intended for quantitative determination of IgG class autoantibodies against Nucleosomes in human serum.

General Description

Nucleosomes are the basic nuclear chromosomal structures that keep DNA in the nucleus compact. The nucleosome is composed of four histamine complexes: H2A, H2B, H3 and H4, and forms a 146bp 8-mer double-stranded DNA. H1 serves as a link between nucleosomes and other nucleosomes linked to DNA.

Anti-double-stranded DNA (dsDNA) and anti-histamine complex antibodies are common features of systemic lupus erythematosus (SLE). In 1993, Burlingame et al. first discovered anti-chromatin antibodies corresponding to T cell-mediated autoantibody immune responses in a mouse model. This study shows that the first autoimmune response in a mouse model of systemic wolf erythematosus (SLE) is directly anti-nucleosome. Therefore, anti-nucleosome-specific antibodies are specific markers for early systemic lupus erythematosus (SLE), which can recognize the antigenic determinants of natural nucleosome molecules. The subsequent autoimmune response is divided into antinucleosome, anti-DNA, and anti-histamine complexes.

The detection rate of nucleosome-specific antibodies in patients with systemic lupus erythematosus (SLE) is 84 to 88%.

Principles of Testing

Human Nucleosomes are bound to microwells. Antibodies against these antigens, if present in diluted serum, bind to the respective antigen. Washing of the microwells removes unspecific serum components. Horseradish peroxidase (HRP) conjugated antihuman IgG immunologically detects the bound specimen sample antibodies forming a conjugate/antibody/ antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG antibodies present in the original sample.

Technical Parameters

1. Due to the lack of international reference standards, the unit of this test result is expressed in U/mL.
2. Test sample: Serum
3. Sample Volume: 10 µL sample diluted 1:101 in 1× Sample Diluent
4. Total incubation time: 20-32°C, 90 minutes
5. Calibrator range: 0-300 U/mL
6. Detection sensitivity: 1.0 U/mL
7. Storage conditions: original packaging, 2-8°C
8. Number of tests: 96 tests

Reagents And Materials Provided

Reagents that need to be prepared:

1. 1 bottle of 5× Sample buffer, 20 mL - 5 times concentrated (white cap: yellow solution)
   Contains: tris (Tris), sodium chloride (NaCl), bovine serum albumin (BSA), azide Sodium <0.1% (preservative)
2. 1 bottle of 50× Washing buffer, 20 mL - 50 times concentrated (white cap: green solution)
   Contains: Tris, sodium chloride (NaCl), Tween 20, stack Nitrogen sodium<0.1% (preservative)

Ready-to-use reagents:

1. Negative quality control 1 bottle, 1.5 mL (green cap: colorless solution)
   Contains: diluted human serum, sodium azide <0.1% (preservative)
2. Positive quality control 1 bottle, 1.5 mL (red cap: yellow solution)
   Contains: diluted human serum, sodium azide <0.1% (preservative)
3. Cut-off quality control 1 bottle, 1.5 mL (blue cap: yellow solution)
   Contains: diluted human serum, sodium azide <0.1% (preservative)
4. Calibrator 6 bottles, each bottle is 1.5 mL, respectively 0, 3, 10, 30, 100, 300 U/mL
   (Color deepens with concentration: yellow solution)
   Contains: diluted human serum, sodium azide <0.1% (preservative)
5. Enzyme conjugate 1 bottle, 15 mL IgG (blue cap: blue solution)
   Contains: Horseradish peroxidase-labeled anti-human immunoglobulin
6. Tetramethylbenzidine (TMB) substrate 1 bottle, 15 mL (black cap)
   Contains: Stabilized Tetramethylbenzidine (TMB)/Hydrogen Peroxide (H_2O_2)
7. Stop solution 1 bottle, 15 mL (white cap: colorless solution)
   Contains: 1 mol/L (M) hydrochloric acid
8. Divisible microplate consisting of 12 modules of 8 wells each, coated with native human Nucleosomes. Ready to use; 1 plate

Materials Required But Not Supplied

1. Microplate reader, measuring wavelength 450 nm, reference wavelength 620 nm (600-690 nm);
2. Glassware (100-1000 mL graduated cylinder), test tube for dilution; mixer;
3. Single-channel sample gun (10, 100, 200, 500, 1000 µL) or Multi-channel sample gun (100-1000 mL);
4. Plate washer (300 µL single-channel or multi-channel flushing or automatic plate washing system);
5. Absorbent paper.

This experiment should use purified water that complies with the United States Pharmacopoeia (USP 26-NF 21) and the European Pharmacopoeia (Eur.Ph. 4th ed.).

Storage

1. Store the kit at 2°C - 8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2°C - 8 °C

Specimen Collection And Preparation

Preferably use freshly collected serum samples. Blood collection should comply with national standards.

Samples with jaundice, lipemia, hemolysis, or bacterial contamination must not be used. Serum containing particulate matter should first be removed by low-speed centrifugation (< 1000 ×g). Blood samples should be collected in clean, dry, empty tubes.

The sample serum should be used immediately after separation. It can be stored in a sealed container at 2-8°C for 3 days, and stored at -20°C for a longer period of time.

Reagent Preparation

Preparation before adding sample:

1. Dilute concentrated reagent (no special instructions, all dilutions in this article are final dilutions):

   Dilute the concentrated sample diluent 5 times with distilled water (for example: 20 mL sample diluent + 80 mL distilled water).

   Dilute the concentrated wash buffer 50-fold with distilled water (for example, 20 mL concentrated wash buffer + 980 mL distilled water).

2. **Sample**

   Dilute the serum sample 101 times with 1× sample diluent; for example: 10 µL serum sample + 1000 µL sample buffer. Mix thoroughly!

3. **Wash buffer**

   Prepare 20 mL of 1× diluted wash buffer for every 8 wells; or 200 mL for 96 wells. For example: add 196 mL deionized water to 4 mL concentrated washing solution.

4. **Automatic flushing**

   It is necessary to refer to the additional dosage of the instrument pipeline and sample adding part for adjustment.

5. **Manual Washing**

   Invert the microwell plate to clear the liquid in the microwells: Invert the microwell plate onto clean absorbent paper, and tap hard to remove the liquid in the microwells; load 300 µL of diluted cleaning buffer into each well and let it stand for 20 seconds; the entire process Repeat 2 times.

6. **Microplate**

   Calculate the number of micropores required for the test, and store unused micropores in the original plastic bag sealed with desiccant (2-8°C).

Assay Procedure

It is recommended that samples and calibrators be run in duplicate.

Critical quality control (CUT-OFF) is only used for qualitative testing.

1. Add 100 µL of diluted patient serum sample to the corresponding microwell.
2. Add 100 µL of negative control, positive control, calibrator or critical control to other corresponding microwells.
3. Incubate at 20-32°C for 30 minutes.
4. Add 300 µL washing buffer (dilution 1:50) to each well and wash 3 times;
5. Add 100 µL of enzyme conjugate to each well;
6. Incubate at 20-32°C for 30 minutes.
7. Add 300 µL washing buffer (dilution 1:50) to each well and wash 3 times;
8. Add 100 µL of tetramethylbenzidine (TMB) substrate solution to each well;
9. Incubate at 20-32°C in the dark for 30 minutes.
10. Add 100 µL stop solution to each well in the same order as adding substrate solution;
11. Incubate for at least 5 minutes;
12. Shake carefully for 5 seconds;
13. Read the absorbance at 450 nm within 30 minutes (450/620 nm optional).

We recommend adding calibrators, controls, and samples according to the following table:

For quantitative experiments, use calibrators to create a calibration curve to interpret the results.

For qualitative experiments, use critical quality controls to interpret results.

	Quantitative						Qualitative					
	1	2	3	4	5	6	7	8	9	10	11	12
A	CalA	CalE	P1				NC	P2				
B	CalA	CalE	P1				NC	P2				
C	CalB	CalF	P2				CC	P3				
D	CalB	CalF	P2				CC	P3				
E	CalC	PC	P3				PC	…				
F	CalC	PC	P3				PC	…				
G	CalD	NC	…				P1	…				
H	CalD	NC	…				P1	…				

Quality Control

All of the following conditions must be met to confirm that the test is valid.

1. Quantitative experiment: Positive and negative quality control and 6 calibrators must be done in each experiment.

   Qualitative experiment: Positive and negative quality control and critical quality control must be done in

every experiment.

2. For details on the QC range of Positive and negative QC, calibrators or critical QC, please see the QC leaflet inside the box.
3. In accordance with the instructions or requirements of the local government or authorized agency, additional quality control may be required.

Calculation

Use the absorbance value (OD) of each calibrator as the Y-axis and the corresponding concentration value (U/mL) as the X-axis to establish a calibration curve for quantitative result interpretation; it is recommended to use the log/lin curve and the 4-parameter method. Use the OD of each sample to read the corresponding antibody concentration value (U/mL) from the calibration curve.

Negative: <12 U/mL

Borderline range: 12-18 U/mL

Positive: >18 U/mL

Interpretation Of Results

Each laboratory should establish its own normal value range according to its own technology, quality controls, equipment, and sample population, following a customized process.

During qualitative experiments, it is necessary to read the absorbance value (OD) of the critical quality control (CUT-OFF) and the patient sample, and obtain the experimental results by comparing the absorbance value (OD) of the patient sample and the critical quality control (CUT-OFF); we recommend that both Samples with a deviation within 20% above and below the critical value are considered suspicious results, samples with a deviation higher than 20% above the critical quality control value are positive results, and samples with a deviation below 20% below the critical quality control value are negative results.

Negative: patient's absorbance value (OD) < 0.8× Cut off absorbance value (OD)

Borderline range: 0.8× Cut off absorbance value (OD) ≤ patient absorbance value (OD) ≤ 1.2× Cut off absorbance value (OD)

Positive: Patient's absorbance value (OD) > 1.2× Cut off absorbance value (OD)

Typical Standard Curve

Calibrator	OD 450/620nm	CV%
0 U/mL	0.030	2.8
3 U/mL	0.136	1.0
10 U/mL	0.339	0.5
30 U/mL	0.661	1.4
100 U/mL	1.255	2.9
300 U/mL	2.131	1.8

Calculation example

Patient	Test Result (OD)	Average (OD)	Result U/mL
1	0.838/0.849	0.844	47.2
2	1.503/1.516	1.510	137.3

Performance Characteristics

1. **Analytical sensitivity**

After testing the sample buffer of this kit 30 times, the analytical sensitivity of this kit is 1.0 U/mL.

2. **Specificity and sensitivity**

The microplate is coated with highly purified natural human nucleosomes, and no cross-reactivity with other autoantigens has been found.

The diagnostic specificity is 90% and the sensitivity is 84-88%.

3. **Response linearity**

The good linearity of this kit test was demonstrated by serial dilutions of selected sera. However, due to the presence of naturally occurring heterogeneous autoantibodies, some samples may not comply with this rule.

	Dilution factor	Test Results U/mL	Expected Results U/mL	Recovery Rate %
1	1/100	152.9	153.6	99.5
	1/200	76.4	76.8	99.4
	1/400	38.8	38.4	101.0
	1/800	17.8	19.2	92.7
2	1/100	85.3	84.9	100.5
	1/200	44.1	42.5	103.8
	1/400	22.0	21.2	103.8
	1/800	9.8	10.6	92.5

Precision

Intra-assay Precision (Precision within an assay) C.V < 10%.

Inter-assay Precision (Precision between assays) C.V < 10%.

Precautions

1. This product is for RUO.

Although this kit has no obvious toxicity or danger in routine use, please follow the following recommendations to maximize your safety:

2. This kit contains potentially toxic ingredients. Although the kit does not contain eye and skin irritants, we still recommend that you wear disposable gloves when using the reagents.
3. **Warning**! Calibrators, quality controls, and buffers use sodium azide as a preservative. Sodium azide may

be toxic if it comes in contact with skin or eyes. Sodium azide can react with lead and copper in pipes to form highly explosive metal azide. Therefore, flush with plenty of water during handling to prevent azide accumulation.

4. Smoking and eating are prohibited when using this reagent. Do not use your mouth to move liquids.
5. All human components in the kit (quality controls, calibrators, etc.) have been tested by recommended methods, and are negative for hepatitis B, hepatitis C, and HIV 1. But there is no way to ensure that such materials are completely free of viral antigens. Therefore, in accordance with regulatory requirements, the controls, calibrators, and patient samples in this kit should be treated as potential sources of infection when using them.
6. Do not mix or replace reagents or microplates from different batch numbers, otherwise it will lead to different results.
7. All reagents should be equilibrated to room temperature (20-32°C) before use, mixed evenly and incubated according to our recommended procedures to achieve the best performance of the reagents.
8. Incubation: When using automated equipment we recommend a test temperature of 30°C.
9. Do not expose reagents to temperatures above 37°C.
10. Use only new tips to pipette substrate solutions; the reagent should be protected from light. Do not use tips that have been used to pipet other reagents to aspirate enzyme conjugates.