Nucleosome Ab ELISA Kit

Catalog Number KA1091

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

Enzyme Immunoassay for quantitative determination of IgG Autoantibodies to Nucleosomes in human serum or plasma

Background

Antibodies directed against nucleosomes were first described in association with systemic lupus erythematosus (SLE) in 1957. At those times known as "LE cell factor". In 1986 Hardin suggested, that nucleosomes possibly were important antigens in generating antinuclear antibodies in SLE-patients. But only in 1995 nucleosomes were properly described as autoantigens in systemic autoimmune diseases. Today, anti-nucleosome antibodies are recognised to be especially prevalent in systemic lupus erythematosus and drug-induced lupus.

Nucleosomes mainly consist of an octamere of histones (four homo-dimers of H2A, H2B, H3, H4) around which 146 bp of DNA are wound twice. Histone H1 interacts with the nucleosome and together with linked-DNA connects neighbouring nucleosomes. Hence the nucleosome structure is important in the compaction of DNA in the nucleus.

Anti-nucleosome-specific antibodies together with lupus anti-dsDNA and anti-histone antibodies directed towards nucleosomes belong to a broad anti-nucleosome antibody family. Systemic lupus erythematosus (SLE) is a chronic multisystemic disease with unknown aetiology. It is characterised by organ damage of vasculitis origin. The main clinical manifestations are renal diseases (50 %), skin rashes (70 %), arthralgia (90 %), involvement of the central nervous system (CNS) (30 %), polyserositis and cytopenia. Due to the difficulty of diagnosing "SLE", 11 criteria were set up by the American College of Rheumatology (ACR), in 1982.

Of the above mentioned 11 criteria, at least 4 must be diagnosed in order to classify an SLE-patient. It could be demonstrated, that anti-nucleosome antibodies are detected in 84 - 88 % of patients with SLE. And a percentage of 16 - 30 % of patients with lupus have been reported to have anti-nucleosome antibodies without anti-dsDNA and anti-histone antibodies.

It has been reported that anti-nucleosome immunglobulin G antibodies are a more sensitive marker of SLE than anti-dsDNA, and are almost exclusively found in lupus, scleroderma, and mixed connective tissue diseases. Furthermore, it has been shown recently, that antinuclear autoantibodies complexed to nucleosomes can bind to heparan sulphate in the glomerular basement membrane (GBM) via the histone part of the nucleosome in SLE nephritis.

Autoantibody prevalence to (values in %)

Diseases	dsDNA	ssDNA	Histone	SS-A	SS-B	Sm	RNP/Sm	Scl-70	Jo-1
Systemic lupus Erythrematosus (SLE)	> 90	> 90	30-50	10-30	30-50	10-30	10-30		
Drug-induced Lupus (DIL)		30-50	50-90						
Sharp-syndrome / mixed connective tissue disease	10-30	10-30					> 90		
Rheumatoid arthritis	10-30	30-50	30-50	10-30					
Sjogren's syndrome	10-30	10-30		> 90	> 90				
Scleroderma	10-30	10-30		10-30				> 90	
Photosensitive dermatitis, dermatomyositis	10-30	10-30							50-90

Principle of the Assay

Human nucleosomes are bound to microwells. Antibodies against the coated antigen, if present in diluted patient sample, bind to the respective antigen. Washing of the microwells removes unbound unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human antibodies immunologically detect the bound patient 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 colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of antibodies present in the original sample.

General Information

Materials Supplied

List of component

Component	Amount
Divisible microplate Consisting of 12 modules of 8 wells each. Ready to use.	96 (8x12) wells
Calibrators A-F (0; 12.5; 25; 50; 100, 200 U/ml), containing serum/ buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use.	6 vials, 1.5 ml each
Control positive (1) and negative (2), containing nucleosome antibodies in a serum/buffer matrix (PBS, BSA, detergent, NaN_3 0.09%), yellow. Ready to use. The concentration is specified on the certificate of analysis.	2 vials, 1,5 ml each
Sample buffer PD, containing PBS, BSA, detergent, preservative NaN_3 0.09%, yellow, 5x conc.	20 ml
Enzyme conjugate containing anti-human IgG antibodies, HRP labeled; PBS, BSA, detergent, preservative ProClin 300 0.05%, light red. Ready to use.	15 ml
TMB substrate; containing 3,3', 5,5'-Tetramethylbenzidin, colorless. Ready to use.	15 ml
Stop solution; contains acid. Ready to use.	15 ml
Wash solution, containing Tris, detergent, preservative NaN_3 0.09%; 50x conc.	20 ml

Storage Instruction

- ✓ Store the kit at 2-8℃ in the dark.
- ✓ Do not expose test reagents to heat, sun or strong light during storage and usage.
- ✓ Store microplate sealed and desiccated in the clip bag provided.
- ✓ Unopened reagents are stable until expiration of the kit. See labels for individual batch.
- ✓ Diluted Wash Solution and Sample Buffer are stable for at least 30 days when stored at 2-8℃. We recommend consumption on the same day.

Materials Required but Not Supplied

- ✓ Microplate reader capable of endpoint measurements at 450 nm: reference filterat 620 nm.
- ✓ Data reduction software
- ✓ Multi-Channel Dispenser or repeatable pipet for 100 µl
- ✓ Vortex mixer
- ✓ Pipets for 10 µl, 100 µl and 1000 µl
- ✓ Laboratory timing device
- ✓ Distilled or deionized water
- ✓ Measuring cylinder for 1000 ml and 100 ml

✓ Plastic container for storage of the wash solution

This ELISA assay is suitable for use on open automated ELISA processors. Each assay has to be validated on the respective automated system. Detailed information is provided upon request.

Precautions for Use

- Procedural Notes

✓ Do not use kit components beyond their expiration dates.
✓ Do not interchange kit components from different lots and products.
✓ All materials must be at room temperature (20-28℃) prior to use.
✓ Prepare all reagents and samples. Once started, perform the test without interruption.
✓ Double determinations may be done. By this means pipetting errors may become obvious.
✓ Perform the assay steps only in the order indicated.
✓ Always use fresh sample dilutions.
✓ Pipette all reagents and samples into the bottom of the wells.
✓ To avoid carryover or contamination, change the pipette tip between samples and different kit controls.
✓ Wash microwells thoroughly and remove the last droplets of wash solution.
✓ All incubation steps must be accurately timed.
✓ Do not re-use microplate wells.

- Warnings and Precautions

✓ All reagents of this kit are intended for professional in vitro diagnostic use only.
✓ Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2 and so all human serum based reagents in this kit must be handled as through capable of transmitting infection.
✓ Bovine serum albumin (BSA) used in components has been tested for BSE and found negative.
✓ Avoid contact with the substrate TMB (3,3',5,5'-Tetramethyl-benzidine).
✓ Stop solution contains acid, classification is non-hazardous. Avoid contact with skin.
✓ Calibrators, Controls, sample buffer and Wash Solution contain sodium azide (NaN_3) 0.09% as preservative. This concentration is classified as non-hazardous.
✓ Enzyme conjugate contains ProClin 300 0.05% as preservative. This concentration is classified as non-hazardous.
✓ During handling of all reagents, controls and serum samples observe the existing regulations for laboratory safety regulations and good laboratory practice:
✓ First aid measures: In case of skin contact, immediately wash thoroughly with water and soap. Remove contaminated clothing and shoes and wash before reuse. If system fluid comes into con-tact with skin, wash thoroughly with water. After contact with the eyes carefully rinse the opened eye with running water for at least 10 minutes. Get medical attention if necessary.
✓ Personal precautions, protective equipment and emergency procedures:

- ✓ Observe laboratory safety regulations. Avoid contact with skin and eyes. Do not swallow. Do not pipette by mouth. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled. When spilled, absorb with an inert material and put the spilled material in an appropriate waste disposal.
- ✓ Exposure controls/ personal protection: Wear protective gloves of nitrile rubber or natural latex. Wear protective glasses. Used according to intended use no dangerous reactions known.
- ✓ Conditions to avoid: Since substrate solution is light-sensitive. Store in the dark.
- ✓ For disposal of laboratory waste the national or regional legislation has to be observed.

Observe the guidelines for performing quality control in medical laboratories by assaying controls sera.

Assay Protocol

Reagent Preparation

- Wash Solution
  Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use.

- Sample Buffer
  Sample Buffer PD Prior to use dilute the contents (20 ml) of one vial of sample buffer 5x concentrate with distilled or deionised water to a volume of 100 ml.

- Preparation of samples
  Dilute patient samples 1:100 before the assay: Put 990 µl of prediluted sample buffer in a polystyrene tube and add 10 µl of sample. Mix well. Note: Calibrators/ Controls are ready to use and need not be diluted.

Sample Preparation

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8℃ for up to five days or stored at -20℃ up to six months.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

Assay Procedure

Prepare enough microplate modules for all calibrators/ controls and patient samples.

1. Pipette 100 µl of calibrators, controls and prediluted patient samples into the wells.
2. Incubate for 30 minutes at room temperature (20-28℃).
3. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
4. Dispense 100 µl of enzyme conjugate into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the microwells and wash 3 times with 300 µl of wash solution.
7. Dispense 100 µl of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature.

9. Add 100 µl of stop solution to each well of the modules.
10. Incubate for 5 minutes at room temperature.
11. Read the optical density at 450 nm (reference 600-690 nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

- Validation

  Test results are valid if the optical densities at 450 nm for calibrators/ controls and the results controls comply with the reference ranges indicated on the Certificate of Analysis enclosed in each test kit. If these quality control criteria are not met the assay run is invalid and should be repeated.

Data Analysis

Calculation of Results

For quantitative results plot the optical density of each calibrator versus the calibrator concentration to create a calibration curve. The concentration of patient samples may then be estimated from the calibration curveby interpolation. Using data reduction software a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Performance Characteristics

- Calibration
  This assay system is calibrated in relative arbitrary units, since no international reference preparation is available for this assay.

- Measuring range
  The calculation range of this ELISA assay is 0-200 U/ml.

- Expected values
  In a normal range study with samples from healthy blood donors the following ranges have been established with this ELISA assay: Cut-off 20 U/ml.

- Interpretation of results
  Negative: $<$ 20 U/ml
  Positive: $\geqq$ 20 U/ml

- Linearity
  Patient samples containing high levels of specific antibody were serially diluted in sample buffer to demonstrate the dynamic range of the assay and upper/ lower end of linearity. Activity for each dilution was calculated from the calibration curve using a 4-Parameter-Fit with lin-log coordinates.

Sample	Dilution	Observe [U/ml]	Expected [U/ml]	O/E [%]
1	1:100	94.3	94.3	100
-	1:200	50.3	47.2	107
-	1:300	23.6	23.6	100
-	1:400	12.4	11.8	105
2	1:500	89.6	89.6	100
-	1:600	43.8	44.8	98
-	1:700	24.0	22.4	107
-	1:800	13.1	11.2	117

- Limit of detection

  Functional sensitivity was determined to be: 0.5 U/ml.

- Interfering Substances

  No interference has been observed with haemolytic (up to 1000 mg/dL) or lipemic (up to 3 g/dL triglycerides) sera or plasma, or bilirubin (up to 40 mg/dL) containing sera or plasma. Nor have any interfering effects been observed with the use of anticoagulants (Citrate, EDTA, Heparine). However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

- Reproducibility

  Intra-Assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 24 determinations in a single run. Results for precision-within-assay are shown in the table below.

  Intra-Assay precision. Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different run. Results for precision-within-assay are shown in the table below.

Intra-Assay			Inter-Assay		
Sample	Mean [U/ml]	CV [%]	Sample	Mean [U/ml]	CV [%]
1	26.0	4.5	1	29.0	12.4
2	61.0	3.1	2	68.0	7.3
3	114.0	6.4	3	138.0	5.2

- Study results

Study population	n	n Pos	%
SLE	110	107	97.3
Rheumatoid Arthritis	20	0	0.0
Normal human sera	200	7	3.5

Clinical Diagnosis

	Pos	Neg	
Pos	107	7	
Neg	3	213	
	110	220	330

Sensitivity: 97.3%

Specificity: 96.8%

Overall agreement: 97.0%

Resources

References

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Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	calibrators	Patient Sample										
B	calibrators	Patient Sample										
C	calibrators	Patient Sample										
D	calibrators											
E	calibrators											
F	calibrators											
G	controls											
H	controls											