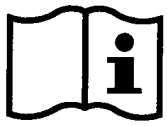
Product information

Information about other products is available at: www.demeditec.com

EN ISO 9001 certified company



Instruction for use

Nucleosome Ab ELISA

Enzyme Immunoassay for Quantitative Determination of IgG Auto-antibodies to Nucleosomes in human serum or plasma

CE

IVD

REF **DE7180**

Σ **96 Tests**

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
Email: info@demeditec.de • http://www.demeditec.com

Version 03-12/12
JS
Updated 130422

PRINCIPLE OF THE TEST

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.

SUMMARY AND EXPLANATION OF THE TEST

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 has been reported to have anti-nucleosome antibodies without anti-dsDNA and anti-histone antibodies.
It has been reported that anti-nucleosome immunoglobulin 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					
Sjögren´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

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
Email: info@demeditec.de • http://www.demeditec.com

CONTENTS OF THE KIT

Sufficient for 96 determinations

1 One divisible microplate consisting of 12 modules of 8 wells each. Ready to use.

6x 1.5 ml Calibrator 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.

2x 1.5 ml 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.

20 ml Sample Buffer PD, containing PBS, BSA, detergent, preservative NaN_3 0.09%, yellow, 5x conc.

15 ml Enzyme Conjugate containing anti-human IgG antibodies, HRP labelled; 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.

20 ml Wash Solution, containing Tris, detergent, preservative NaN_3 0.09%; 50 x conc.

1 Instruction for Use

1 Certificate of Analysis

MATERIALS REQUIRED

- Microplate reader capable of endpoint measurements at 450 nm; optional: reference filter at 620 nm
- Data reduction software
- Multi-channel dispenser or repeatable pipette for 100 µl
- Vortex mixer
- Pipettes for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Distilled or deionised 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.

SPECIMEN COLLECTION, STORAGE AND HANDLING

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

STORAGE AND STABILITY

- Store test kit at 2-8°C in the dark.
- Do not expose 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°C. We recommend consumption on the same day.

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
Email: info@demeditec.de • http://www.demeditec.com

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°C) 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 though 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 contact 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 control sera.

PREPARATION OF REAGENTS

Wash Solution
Dilute the contents of one vial of the buffered wash solution concentrate (50x) with distilled or deionised 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 final 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.

TEST 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 °C).
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 600-690nm) and calculate the results. The developed colour is stable for at least 30 minutes. Read during this time.

Example for a pipetting scheme:

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	P1										
B	B	P2										
C	C	P3										
D	D											
E	E											
F	F											
G	C+											
H	C-											

P1, ... patient sample A-F calibrators C+, C- controls

VALIDATION

Test results are valid if the optical densities at 450 nm for calibrators / controls and the results for 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.

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 curve by 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: ≥ 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 the 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	Observed U/ml	Expected U/ml	O/E [%]
1	1:100	94.3	94.3	100
.	1:200	50.3	47.2	107
.	1:400	23.6	23.6	100
.	1:800	12.4	11.8	105
2	1:100	89.6	89.6	100
.	1:200	43.8	44.8	98
.	1:400	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.
Inter-assay precision: Coefficient of variation (CV) was calculated for each of three samples from the results of 6 determinations in 5 different runs. Results for run-to-run precision are shown in the table below.

Intra-Assay		
Sample	Mean U/ml	CV %
1	26.0	4.5
2	61.0	3.1
3	114.0	6.4

Inter-Assay		
Sample	Mean U/ml	CV %
1	29.0	12.4
2	68.0	7.3
3	138.0	5.2

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
Email: info@demeditec.de • http://www.demeditec.com

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 %

LIMITATIONS OF THE PROCEDURE

This assay is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated concerning the entire clinical picture of the patient. Also every decision for therapy should be taken individually.
The above pathological and normal reference ranges for antibodies in patient samples should be regarded as recommendations only. Each laboratory should establish its own ranges according to ISO 15189 or other applicable laboratory guidelines.

REFERENCES

1. Burlingame RW, Boey ML, Starkebaum G, et al. The central role of chromatin in autoimmune responses to histones and DNA in systemic lupus erythematosus. J Clin Invest, Jul 1994, 94(1) p184-92
2. Hardin J. A. The lupus autoantigens and the pathogenesis of systemic lupus erythematosus. Arthritis Rheum, Apr 1986, 29 (4) p457-60
3. Amital H, Shoenfeld Y. Nucleosomes, DNA and SLE: where is the starting point? [editorial; comment]. Clin Exp Rheumatol, Sep-Oct 1996, 14 (5) p475-7
4. Burlingame R. W. The clinical utility of antihistone antibodies. Autoantibodies reactive with chromatin in systemic lupus erythematosus and drug-induced lupus. Clin Lab Med, Sep 1997, 17(3) p367-78
5. Berden J. H., Licht R., van Bruggen M. C., et al. Role of nucleosomes for induction and glomerular binding of autoantibodies in lupus nephritis. Curr Opin Nephrol Hypertens, May 1999, 8(3) p299-306
6. Amoura Z., Koutouzov S., Chabre H., et al. Presence of anti-nucleosome autoantibodies in a restricted set of connective tissue diseases: anti-nucleosome antibodies of the IgG3 subclass are markers of renal pathogenicity in systemic lupus erythematosus. Arthritis Rheum, Jan 2000, 43(1) p76-84
7. Chabre H., Amoura Z., Piette J. C., et al. Presence of nucleosome-restricted antibodies in patients with systemic lupus erythematosus. Arthritis Rheum, Oct 1995, 38.(10) p1485-91
8. Holman HR, Kunkel HG Affinity between the lupus erythematosus serum factor and cell nuclei and nucleoprotein. Science, 1957, 126 p.162-3

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
Email: info@demeditec.de • http://www.demeditec.com

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • D-24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
Email: info@demeditec.de • http://www.demeditec.com