DRG® Anti-Nucleosome (EIA-3610)

Revised 1 Apr. 2010 rm (Vers. 2.0) **RUO in the USA**

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

NAME AND INTENDED USE

Anti-Nucleosome is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against Nucleosomes in human serum or plasma.
The assay is intended for in vitro use only as an aid in the determination of Systemic Lupus Erythematosus (SLE).

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:

– Malar rashes	on both cheeks
– Discoid rush	erythematous raised patches
– Photosensitivity	skin rash as a result of unusual reaction to sunlight
– Oral ulcers	oral or nasopharyngeal ulceration, usually painless
– Arthritis	nonerosive arthritis involving 2 or more peripheral joints
– Serositis	documented pleuritis or pericarditis
– Renal disorder	persistent proteinuri > 0.5 g/day or cellular casts
– Neurologic disorder	seizures or psychosis
– Haematological disorder	haemolytic anaemia or leukopenia or lymphopenia or thrombocytopenia
– Immunologic disorder	positive LE cell preparation or anti-dsDNA antibodies or anti-Sm antibodies or false positive serologic test for syphilis
– Antinuclear antibody	an abnormal titre of antinuclear antibody in the absence of drugs known to be associated with "drug-induced lupus" syndrome

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.

PRINCIPLE OF THE TEST

Human Nucleosomes are bound to microwells. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated antihuman IgG immunologically detects 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 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.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro use only.
2. Do not interchange kit components from different lots.
3. 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 and HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3´,5,5´-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

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CONTENTS OF THE KIT

Package size	96 determinations
Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with human Nucleosomes. Ready to use.
6 vials, 1.5 ml each	combined Calibrators with IgG class Anti- Nucleosome antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN_3 <0.1% (w/w)) containing: IgG: 0; 12.5; 25; 50; 100; and 200 U/ml. Ready to use.
2 vials, 1.5 ml each	Anti- Nucleosome Controls in a serum/buffer matrix (PBS, BSA, NaN_3 <0.1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed QC insert. Ready to use.
1 vial, 20 ml	Sample buffer (Tris, NaN_3 <0.1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	Enzyme conjugate solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution. Ready to use.
1 vial, 15 ml	Stop solution (contains acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, NaN_3 <0.1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8 °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 2-8 °C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 µl
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl
- Laboratory timing device
- Data reduction software

Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

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SPECIMEN COLLECTION, STORAGE AND HANDLING

- Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
- Allow blood to clot and separate the serum by centrifugation.
- Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best 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 samples. This may result in variable loss of autoantibody activity.
- Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).
4. Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use.
Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use.
Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples **1:100** with sample buffer before assay.
Therefore combine 10 µl of sample with 990 µl of sample buffer in a polystyrene tube. Mix well.
Controls are ready to use and need not be diluted.

TEST PROCEDURE

1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipet **100 µl** of calibrators, controls and prediluted patient samples in duplicate into the wells.

	1	2	3	4	5	6
A	SA	SE	P1	P5		
B	SA	SE	P1	P5		
C	SB	SF	P2	P..		
D	SB	SF	P2	P..		
E	SC	C1	P3			
F	SC	C1	P3			
G	SD	C2	P4			
H	SD	C2	P4			

SA-SF: standards A to F
P1, P2...: patient sample 1, 2 ...
C1: positive control
C2: negative control

3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash **3 times** with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash **3 times** with **300 µl** of wash solution.
8. Dispense **100 µl** of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature.

11. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Automation
The Anti-Nucleosome ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Positive Control (1) and Negative Control (2) as well as for the Calibrator A and F complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit ! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Calculation of results

For Anti-Nucleosome IgG a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot
First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Calculation example
The figures below show typical results for Anti- Nucleosome ELISA. These data are intended for illustration only and should not be used to calculate results from another run.

Calibrators									
No	Position	OD 1	OD 2	Mean	Conc. 1	Conc. 2	Mean	decl. Conc.	CV %
ST1	A 1/A 2	0.023	0.021	0.022	0.0	0.0	0.0	0,0	9
ST2	B 1/B 2	0.174	0.174	0.174	13	13	13	12	0
ST3	C 1/C 2	0.336	0.335	0.336	25	25	25	25	0
ST4	D 1/D 2	0.643	0.658	0.651	49	50	50	50	2
ST5	E 1/E 2	1.172	1.173	1.173	101	101	101	100	0
ST6	F 1/F 2	1.809	1.788	1.799	201	197	199	200	1

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti- Nucleosome test:

	Anti- Nucleosome [U/ml]
Cut-Off:	20

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges. The values above should be regarded as guidelines only.

PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-Nucleosomes kit. The assay shows linearity over the full measuring range.

Precision (Reproducibility)

Statistics were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations each:

Intra-Assay		
Sample No	Mean (U/ml)	CV (%)
1	26	4,5
2	61	3,1
3	114	6,4

Inter-Assay		
Sample No	Mean (U/ml)	CV (%)
1	29	12,4
2	68	7,3
3	138	5,2

Sensitivity

The lower detection limit for Anti-Nucleosomes has been determined at 1.0 U/ml.

Specificity

The solid phase is coated with human nucleosomes. Therefore the Anti-Nucleosome ELISA recognizes only autoantibodies directed against nucleosomes.

Calibration

Since no international reference preparation for anti-nucleosome autoantibodies is available, the assay system is calibrated in relative arbitrary units.

LIMITATIONS OF PROCEDURE

The Anti-Nucleosome ELISA 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.

INTERFERING SUBSTANCES

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

REFERENCES / Literature

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Symbols used with DRG 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-Konfirmitäts-kennzeichnung	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
Σ	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	Mindesthaltbarkeits-datum	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
Σ		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	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..