



# INSTRUCTION MANUAL

REF 4001

June 9, 2008

## Anti-Nucleo

- 96 determinations -



IVD *In vitro* diagnostic device

Enzyme immunoassay for the determination of IgG antibodies to nucleosomes in human serum

<b>REF</b>	Catalogue number	<b>LOT</b>	Batch code
	Consult accompanying documents		Manufactured by
	Temperature limitation		Use by
	Consult operating instruction		Biological risk



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### INTENDED USE

**Anti-Nucleo is used for the quantitative determination of IgG autoantibodies to nucleosomes and their components in patients with Systemic Lupus Erythematosus (SLE) in human serum.**

SLE is an autoimmune disease characterized by the occurrence of numerous antibodies against chromatin antigens, notably dsDNA and histones.

The nature of autoantigens that initiate the production of autoantibodies to these chromatin components has been obscure so far. Madaio et al.<sup>1</sup> (1984) proved mammalian DNA to be not immunogenic. Furthermore, an immunization with mammalian DNA does not induce pathogenic anti-DNA antibodies that cause clinical symptoms.

Burlingame et al.<sup>2</sup> (1993) were the first to show in murine models that the production of anti-chromatin antibodies is induced by a T-cell dependent immune response.

Recent studies demonstrated that the initial autoimmune response in murine models of SLE is directed against nucleosomes being the structural unit of chromatin, an association of dsDNA and histones.

Thus anti-nucleosome antibodies represent specific and early markers of SLE recognizing conformational epitopes shared by the native nucleosome molecule. Later the autoimmune response can diversify to the components of the nucleosomes, DNA and histones, as part of intermolecular spreading.

Moreover, nucleosome and anti-nucleosome complexes have been identified as the major pathogenic agent in lupus nephritis as they are deposited especially in the renal glomerular basement membrane. Anti-nucleosome antibodies seem to be more specific and early markers of lupus nephritis than dsDNA antibodies.

The detection of anti-nucleosome antibodies might be considered as the first step in the diagnosis of SLE as they appear to represent one of the earliest, detectable marker of this autoimmune disease.

Anti-Nucleo allows the determination of autoantibodies to all three chromatin components: **nucleosomes**, **histones** and **dsDNA**, thus being the most specific diagnostic tool for the differential diagnosis of SLE and its pathological variants.

- MADAILO MP, HODDER S, SCHWARTZ RS et al.: Responsiveness of autoimmune and normal mice to nucleic acid antigens. J Immunol 132 1984, 872-882
- BURLINGAME, RW, RUBIN RL, BALDERAS RS, THEOFILOPOULOS AN: Genesis and evolution of antichromatin autoantibodies in murine lupus indicates T-dependent immunization with self antigen. J Clin Invest 91 1993, 1687-1696

### TEST PRINCIPLE

Anti-Nucleo is an enzyme immunoassay for the quantitative determination of autoantibodies to nucleosomes and their components in human serum.

Autoantibodies of the diluted patient samples, control, and calibrators react with nucleosome antigen immobilized on the solid phase of a microtiter plate. Anti-Nucleo guarantees the specific binding of nucleosomal autoantibodies of the specimen under investigation by employing a highly purified nucleosome antigen for coating. Following an incubation period of 60 min at room temperature (18...25°C), unbound sample components are removed by a wash step.

The bound IgG antibodies react specifically with anti-human-IgG conjugated to horseradish peroxidase (HRP). Within the incubation period of 30 min at room temperature, excessive conjugate is separated from the solid-phase immune complexes by the following wash step.

HRP converts the colorless substrate solution of 3,3',5,5'-tetramethylbenzidine (TMB) added into a blue product. The enzyme reaction is stopped by dispensing an acidic solution into the wells after 15 min at room temperature turning the solution from blue to yellow.

The optical density (OD) of the solution at 450 nm is directly proportional to the amount of specific antibodies bound. The standard curve is established by plotting the concentrations of the antibodies of the calibrators (x-axis) and their corresponding OD values (y-axis) measured. The concentration of antibodies of the specimen is directly read off the standard curve.

## PATIENT SAMPLES

### Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. EDTA- and Heparin Plasma (**no citrate**) is also suitable for use in Anti-Nucleo. Lipaemic, hemolytic and contaminated samples should not be used.

Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at 20 °C.

### Preparation before use

Allow samples to reach room temperature prior to assay. Take care to agitate serum samples gently in order to ensure homogeneity.

**Note:** *Patient samples have to be diluted 1 + 100 (v/v), e.g. 10 µl sample + 1 ml sample diluent (C), prior to assay.*

The samples may be kept at 2 - 8 °C for up to two days. Long-term storage requires - 20 °C.

## TEST COMPONENTS for 96 determinations

<b>A</b> <b>Ag</b> <b>96</b>	<b>Microtiter plate</b> , 12 breakable strips per 8 wells (total 96 individual wells) coated with nucleosome antigen (avian)	1 sealed in a plastic foil
<b>B</b> <b>BUF</b> <b>WASH</b> <b>10x</b>	<b>Wash buffer</b> sufficient 1000 ml solution	100 ml concentrate (capped white)
<b>C</b> <b>DIL</b>	<b>Sample diluent</b>	100 ml ready for use (capped black)
<b>D</b> <b>CONJ</b>	<b>Conjugate</b> containing anti-human-IgG (rabbit) coupled with HRP	15 ml ready for use (capped red)
<b>E</b> <b>SOLN</b> <b>TMB</b>	<b>Substrate</b> 3,3',5,5'-tetramethylbenzidine in citrate buffer containing hydrogen peroxide	15 ml ready for use (capped blue)
<b>F</b> <b>H2SO4</b> <b>0.25M</b>	<b>Stop solution</b> 0.25 M sulfuric acid	15 ml ready for use (capped yellow)
<b>0 - 4</b> <b>CAL</b>	<b>Calibrators</b> (diluted serum) conc.: 1, 10, 30, 100, 300 U/ml	1 ml each ready for use capped white
<b>P</b> <b>CONTROL</b>	<b>Positive control</b> (diluted serum) conc.: see leaflet enclosed <b>+</b>	1 ml ready for use capped red

### Materials required

- micropipette 100 - 1000 µl
- micropipette 10 - 100 µl
- multi-channel pipette 50 - 200 µl
- trough for multi-channel pipette
- 8-channel wash comb with vacuum pump and waste bottle or microplate washer
- microplate reader with optical filters for 450 nm and 620 nm or 690 nm
- graduated cylinders
- distilled or de-ionized water

### Size and storage

Anti-Nucleo has been designed for 96 determinations.

The expiry date of each component is reported on its respective label that of the complete kit on the box labels.

Upon receipt, all components of the Anti-Nucleo have to be kept at 2 - 8 °C, preferably in the original kit box.

After opening all kit components are stable for at least 2 months, provided proper storage.

### Preparation before use

Allow all components to reach room temperature prior to use in the assay.

The microtiter plate is sealed in a plastic foil. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original cover carefully resealed.

Prepare a sufficient amount of wash solution by diluting the concentrated wash buffer 10 times (1 + 9) with de-ionized or distilled water. For example, dilute 8 ml of the concentrate with 72 ml of distilled water per strip. The wash solution prepared is stable at 2-8 °C up to 30 days.

Make sure the soak time of the wash buffer in the wells is at least 5 seconds per wash cycle.

Avoid exposure of the TMB substrate solution to light!

## ASSAY PROCEDURE

- Dilute patient sera with sample diluent (C) 1 + 100 (v/v), e.g. 10 µl serum + 1 ml sample diluent (C).
- Avoid any time shift during pipetting of reagents and samples.

1. Bring all reagents to room temperature (18...25°C) before use. Mix gently without causing foam.
2. Dispense  
**100 µl** calibrators 1 – 4 (CAL 0 optionally)  
**100 µl** positive control (P)  
**100 µl** diluted patient samples into the respective wells.
3. Cover plate, incubate **60 min** at room temperature (18...25°C).
4. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
5. Add **100 µl** of conjugate (D) solution to each well.
6. Cover plate, incubate **30 min** at room temperature (18...25°C).
7. Decant, then wash each well **three** times using **300 µl** wash solution (made of B).
8. Add **100 µl** of substrate (E) to each well.
9. Cover plate, incubate **15 min protected from light** at room temperature (18...25°C).
10. Add **100 µl** of stop solution (F) to each well and mix gently.
11. Read the OD at **450 nm** versus 620 or 690 nm within 30 min after adding the stop solution.

## DATA PROCESSING

### We recommend lin / log processing for best results.

The standard curve is established by plotting the mean OD-values of the calibrators 1 - 4 (CAL 0 optionally) on the ordinate, y-axis, versus their respective anti-nucleosome-concentrations on the abscissa, x-axis, (log. scale).

Nucleosome concentrations of the unknown samples are directly read off in U/ml against the respective OD values.

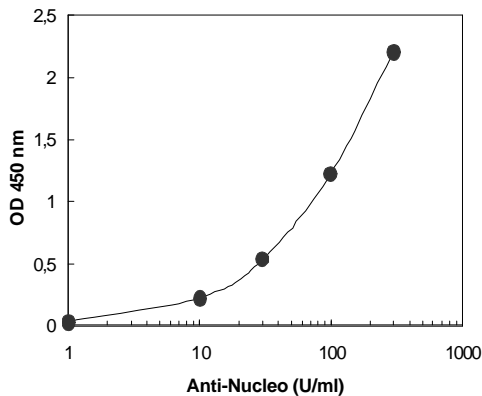
Using the recommended dilution of 1 + 100 (v/v) for patient's sera, no correction factor is necessary, as all other components of the kit are supplied accordingly.

Anti-Nucleo may be used also with Computer Assisted Analysis using software able to plot lin/lin curves with point-to-point fit.

### Example of typical assay results

well	OD (a)	OD (b)	OD (mean)	U/ml
Calibrator 0	0.030	0.036	0.033	1
Calibrator 1	0.230	0.220	0.225	10
Calibrator 2	0.524	0.542	0.533	30
Calibrator 3	1.248	1.199	1.224	100
Calibrator 4	2.234	2.182	2.208	300
Patient 1	0.435	0.385	0.410	21

### STANDARD CURVE (Example)



Specimens with an OD > calibrator 4 should be retested in a greater sample dilution. The results have to be multiplied with the chosen dilution factor.

### Test validity

The test run is valid if:

- the mean OD of the calibrator 1 is  $\leq 0.5$
- the mean OD of the calibrator 4 is  $\geq 1.2$

If the above mentioned quality criteria are not met, repeat the test and make sure that the test procedure is followed correctly (incubation times and temperatures, sample and wash buffer dilution, wash steps etc.). In case of repeated failure of the quality criteria contact your supplier.

## REFERENCE VALUES

Anti-Nucleo	U/ml
positive	> 50
negative	< 30
borderline	30 – 50

## Limitations of Method

Healthy individuals should be tested negative by the Anti-Nucleo. However, nucleosome autoantibody positive apparently healthy persons do occur.

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

## CHARACTERISTIC ASSAY DATA

### Calibration

Due to the lack of an international reference material the Anti-Nucleo is calibrated in arbitrary units (U/ml).

### Linearity

Dilutions of specimens in nucleosome autoantibody free human serum are determined according to their expected theoretical values with Anti-Nucleo.

### Diagnostic Specificity and Sensitivity

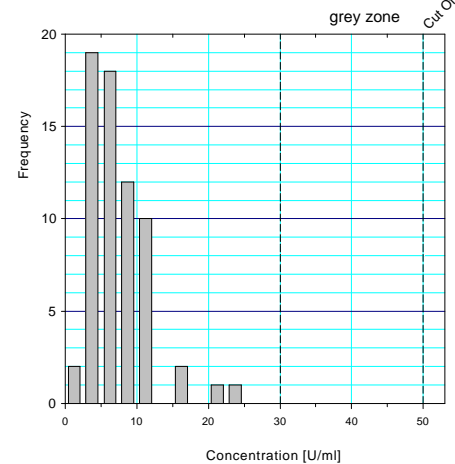
Specificity and sensitivity data of the Anti-Nucleo have been determined by investigating 100 blood donors, 100 patients with chronic inflammatory disorders and 70 patients with SLE.

Specificity: 99 %

Sensitivity: 78 %

### Frequency distribution

65 normal sera were tested in Anti-Nucleo. All sera were found negative. This corresponds to a specificity of 100%.



### Precision

Intraassay coefficient of variation (CV) in Anti-Nucleo (n = 8):

Sample	U/ml	CV (%)
serum 1	202.0	7.1
serum 2	132.3	6.0
serum 3	69.4	3.3
serum 4	48.5	2.3

Interassay coefficient of variation (CV) in Anti-Nucleo (n = 8 x 4):

Sample	U/ml	CV (%)
serum A	200.9	9.6
serum B	127.2	8.6
serum C	66.9	6.6
serum D	39.3	6.0

## INCUBATION SCHEME

# Anti-Nucleo (4001)

**Dilute patients sample★      10 µl serum + 1.0 ml sample diluent (C)**

1	Bring all ready for use reagents to room temperature (18...25°C) before use.			
		calibrators	control	sera
2	Pipette Calibrators (0 - 4) Positive Control (P) prediluted 1 + 100 patient sera	100 µl	100 µl	100 µl
3	Incubate      60 minutes at room temperature (18...25°C)			
4	Wash      Decant, Dispense 3 x 300 µl (made of B)			
5	Pipette conjugate (D)	100 µl	100 µl	100 µl
6	Incubate      30 minutes at room temperature (18...25°C)			
7	Wash      Decant, Dispense 3 x 300 µl (made of B)			
8	Pipette substrate (E)	100 µl	100 µl	100 µl
9	Incubate protected from light      15 minutes at room temperature (18...25°C)			
10	Pipette stop solution (F)	100 µl	100 µl	100 µl
11	Measure 450 nm versus 620 (690) nm			

## SAFETY PRECAUTIONS

- **This kit is for in vitro use only.** Follow the working instructions carefully. GA GENERIC ASSAYS GmbH and its authorized distributors shall not be liable for damages indirectly or consequentially brought about by changing or modifying the procedure indicated. The kit should be performed by trained technical staff only.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for reconstituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Thimerosal (< 0.1 % w/v) and Kathon (1.0 % v/v) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.