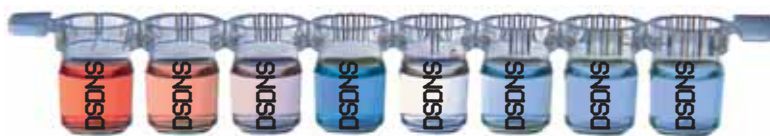




Anti-dsDNA-NcX ELISA (IgG)



Indication: Test system for the in vitro determination of antibodies against dsDNA in human serum or plasma for the diagnosis of the following disease: systemic lupus erythematosus (SLE).

Clinical significance: Antibodies against DNA are distinguished into two different types: Antibodies against double-stranded, native DNA (dsDNA) and antibodies against single-stranded, denatured DNA. Antibodies against dsDNA react mainly with epitopes in the deoxyribose phosphate backbone of the double helix. On the other hand, antibodies defined as reactive with ssDNA recognize epitopes of purine and pyrimidine bases but they may also react with epitopes of the deoxyribose phosphate backbone. Antibodies against dsDNA are the main focus in the serological diagnosis of systemic lupus erythematosus (SLE). These antibodies can be found in 60% to 90% of patients, depending on the activity of the disease. Antibodies against nucleosomes are also an exclusive marker of SLE, provided that they are determined using a perfect test system whose target antigen must be free of histone H1, Scl-70 and other non-histone proteins.

Application of the Anti-dsDNA-NcX ELISA: Using an innovative biochemical preparation, researchers of EUROIMMUN AG have developed a new test system that exceeds the diagnostic quality criteria of all conventional Anti-dsDNA ELISA by far (Anti-dsDNA-NcX ELISA, EUROIMMUN AG, Luebeck, Germany). The secret of the innovation lies in the use of highly purified nucleosomes as the new linking substance. Since nucleosomes have a strong adhesive ability, even the smallest concentration of these is highly suited to couple isolated dsDNA to the surface of a microplate well. Poly-L-lysine and protamine sulphate have fallen into disuse, many false positive results are prevented, the specificity of the ELISA amounts to values which equal those of the indirect immunofluorescence test using *Crithidia luciliae*!

In a clinical comparison study using 378 samples from patients with rheumatic diseases (209 of these with SLE) the Anti-dsDNA-NcX ELISA clearly demonstrated its superiority over the Anti-dsDNA RIA (Farr assay), showing an 8% higher sensitivity.

| Panel (Source: Charité Universitätsmedizin Berlin) | n | Anti-dsDNA NcX- ELISA positive | Anti-dsDNA RIA positive | Anti-dsDNA ELISA positive | IIFT (<i>Crithidia luciliae</i>) positive |
|---------------------------------------------------------------|------|-----------------------------------|----------------------------|------------------------------|------------------------------------------------|
| SLE | 209* | 125 | 108 | 88 | 57 |
| Sensitivity | 209 | 59.8% | 51.7% | 42.1% | 27.4% |
| Sjögren's syndrome | 88 | 1 | 0 | 1 | 1 |
| Progressive systemic sclerosis | 81 | 2 | 2 | 4 | 6 |
| Specificity | 169 | 98.2% | 98.8% | 97.0% | 95.9% |
| Sensitivity at 98% specificity (according to ROC analysis) | 378 | 60.8% | 53.1% | 35.4% | – |

* Only 208 SLE sera were incubated on *Crithidia luciliae*

The Anti-dsDNA-NcX ELISA can be performed manually or fully automatically (EUROIMMUN Analyzer I). Other competent test systems such as the Farr assay and the indirect immunofluorescence test (substrate *Crithidia luciliae*) continue to be of importance in the clarification of discrepant serological and clinical results.

EUROIMMUN Microplate ELISA

Autoantibody determination:

AMA M2-3E (IgG)
ANCA Profile (IgG)
ANA Screen (IgG)
ANA Screen 9* or 11* (IgG)
ANA VarioProfile (IgG)
BP180-4X (IgG)
C1q (IgG)
cardiolipin (IgA, IgG, IgM, IgAGM)
circulating immune complexes (CIC)
cyclic citrullinated peptide (CCP; IgG)
centromere protein B (IgG)
double-stranded DNA (dsDNA, nDNA; IgG)
dsDNA-NcX (IgG)
ENA Pool* (IgG)
ENA PoolPlus (IgG)
ENA ProfilePlus 1 or 2 (IgG)
ENA SLE Profile 1 or 2 (IgG)
GAD
GAD/IA-2 Pool
glomerular basement membrane (GBM; IgG)
β2-glycoprotein 1 (IgA, IgG, IgM, IgAGM)
histones (IgG)
IA-2
intrinsic factor (IgG)
Jo-1 (IgG)
liver cytosolic antigen type 1 (LC-1; IgG)
liver-kidney microsomes (LKM-1; IgG)
myeloperoxidase (MPO; IgG)
nRNP/Sm (IgG)
nucleosomes (IgG)
p53 (IgG)
parietal cells (PCA; IgG)
PM-Scl (PM-1; IgG)
phosphatidylserine (IgA, IgG, IgM, IgAGM)
proteinase 3 (IgG)
PR3 hn-hr (IgG)
PR3 capture (IgG)
rheumatoid factor (IgA, IgG, IgM)
ribosomal P-proteins (IgG)
Scl-70 (IgG)
single-stranded DNA (ssDNA; IgG)
SLA/LP (IgG)
Sm (IgG)
SS-A (Ro; IgG)
SS-B (La; IgG)
thyroglobulin (TG; IgG)
thyroid peroxidase (TPO; IgG)
tissue transglutaminase (endomy; IgA, IgG)
TSH receptor (TBI; IgG)
TRAK Fast (IgG)

Further autoimmune diagnostics:

GAF-3X (IgA, IgG)
gliadin (IgA, IgG)
Saccharomyces cerevisiae (IgA, IgG)

Infectious serology:

Adenovirus (IgA, IgG, IgM)
Borrelia (IgG, IgM)
Borrelia VlsE (IgG)
Chlamydia pneumoniae (IgA, IgG, IgM)
Chlamydia trachomatis (IgA, IgG, IgM)
Cytomegalovirus (IgG, IgM)
Diphtheria toxoid (IgG)
Epstein-Barr virus capsid ag (IgA, IgG, IgM)
Epstein-Barr virus early ag (IgA, IgG, IgM)
Epstein-Barr virus nuclear ag, EBNA-1 (IgG)
Helicobacter pylori (IgA, IgG)
Helicobacter pylori CagA (IgA, IgG)
HSV-1 glycoprotein C1; (IgA, IgG, IgM)
HSV-2 glycoprotein G2; (IgA, IgG, IgM)
HSV-1/2 Pool (IgA, IgG, IgM)
Influenza virus type A (IgA, IgG, IgM)
Influenza virus type B (IgA, IgG, IgM)
Legionella pneumophila (IgA, IgG, IgM)
Measles virus (IgG, IgM)
Mumps virus (IgG, IgM)
Mycoplasma pneumoniae (IgA, IgG, IgM)
Parainfluenza virus Pool (IgA, IgG, IgM)
RSV (IgA, IgG, IgM)
Rubella virus (IgG, IgM)
SARS-CoV (IgG)
TBE virus (IgG, IgM)
Tetanus toxoid (IgG)
Toxoplasma gondii (IgG, IgM)
Treponema pallidum (IgG, IgM)
Varicella zoster virus (IgG, IgM)
Yersinia enterocol. virulence fact. (IgA, IgG)

Allergology:

total IgE
Allercoast™ 6-ELISA (600 different allergens and allergen mixtures)

Serum proteins and tumour markers:
anti-p53

* Currently not available as IVD in the EU.

Made in Germany



EUROIMMUN Immunoblots

Autoantibody determination:

EUROASSAY:

flexible profiles of up to 7 antigens from:

ENA and related antigens: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, dsDNA, histones, nucleosomes, CENP B, PM-Scl, ribosomal P-proteins, AMA M2

liver antigens: LKM-1, LC-1, SLA/LP, AMA M2, M4, M5

ANCA antigens: MPO, PR3

thyroid antigens: TG, TPO

EUROLINE:

ANA Profile 1: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1, CENP B, dsDNA, nucleosomes, histones, ribosomal P-proteins

ANA Profile 3: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, PM-Scl, Jo-1, CENP B, PCNA, dsDNA, nucleosomes, histones, ribosomal P-proteins, AMA M2

Anti-ENA Profile 1: nRNP/Sm, Sm, SS-A, Ro-52, SS-B, Scl-70, Jo-1

Myositis Profile: Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12, Ro-52

Liver Profiles: AMA M2, 3E (BPO), Sp100, PML, gp210, LKM-1, LC-1, SLA/LP, Ro-52

Neuronal Antigens Profile: amphiphysin, CV2/CRMP5, PNM2A2 (Ma-2), Ri, Yo, Hu

Anti-Ganglioside Profile 1: GM1, GD1b, GQ1b

Anti-Ganglioside Profile 2: GM1, GM2, GM3, GD1a, GD1b, GT1b, GQ1b

ANCA Profiles: MPO, PR3, GBM

EUROLINE-WB:

liver-specific antigens (+ recomb. SLA/LP)
neuronal antigens (+ recomb. Hu, Yo, Ri)
HEp-2 cell antigens (+ SS-A and Ro-52, CENP B)
Myositis ag (Mi-2, Ku, PM-Scl, Jo-1, PL-7, PL-12)

Infectious serology:

EUROLINE:

EBV Profile (IgG, IgM, VCA gp125, VCA p19 and EBNA-1, p22, EA-D)
TORCH Profile* (T. gond., rubella, CMV, HSV-1, -2)
Malaria Profile 1: Plasmodium falciparum HRP2 and MSP2, Plasmodium vivax MSP and CSP

Westemblot:

Borrelia burgdorferi (IgG, IgM)
Borrelia afzelii (IgG, IgM)
Borrelia garinii (IgG, IgM)
Epstein-Barr virus (IgG, IgM)
Helicobacter pylori (IgA, IgG)
Treponema pallidum (IgG, IgM)
Yersinia enterocol. virulence fact. (IgA, IgG)

EUROLINE-WB:

Anti-Borrelia (B. afzelii + rec. VlsE)
Anti-HSV (HSV-1 + HSV-2 gG2)
Treponema pallidum + cardiolipin

Allergology:

EUROASSAY:

Domestic Animal Profile (IgE)
Food Profile (IgE)
Inhalation Profile (IgE)
Insect Venom Profile (IgE)
Latex Profile (IgE)
Latex plus Profile (with ficus and fruit; IgE)

EUROLINE:

Atopy Profile (IgE)
Food Profile (IgE)
Inhalation Profile (IgE)
Paediatric Inhalation Profile
Pollen-Food Cross Reaction Profile (IgE)

Software/Automation:

EUROLineScan
camera system EUROBlotCamera
scanner system EUROBlotScanner
incubation processor EUROBlotMaster

EUROIMMUN

Radioimmunoassays

Autoantibody determination:

thyroid peroxidase (TPO; IgG)
thyroglobulin (TG; IgG)
TSH receptor (IgG)
acetylcholine receptor (AChR; IgG)
glutamic acid decarboxylase (GAD; IgG)
insulin (IAA; IgG)
P/Q calcium channel* (VGCC; IgG)
tyrosine phosphatase (IA2; IgG)
dsDNA (IgA/IgG/IgM)

Antigen determination:

thyroglobulin (TG)

Hormone determination:

free triiodothyronine (FT3)
free thyroxine (FT4)
thyrotropin (TSH)
calcitonin

* Currently not available as IVD in the EU.

Made in Germany

Version: 12/07
EA_1572_D_UK_A02

Test characteristics Anti-dsDNA-NcX ELISA (IgG)

Linearity: The linearity of the Anti-dsDNA-NcX ELISA (IgG) was determined by assaying 8 serial dilutions of 4 serum samples. The linear regression was calculated, R2 amounting to >0.95 in all samples. The Anti-dsDNA-NcX ELISA (IgG) is linear at least in the tested concentration range of 40 RU/ml to 757 RU/ml.

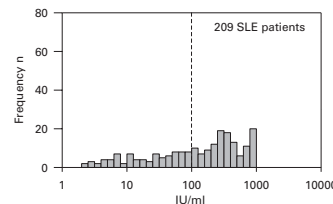
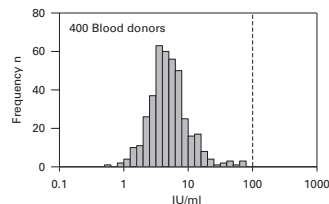
Reproducibility: The reproducibility of the test was investigated by determining the intra- and inter-assay coefficients of variation using 4 sera. The intra-assay CVs are based on 20 determinations and the inter-assay CVs on 4 determinations performed in 6 different test runs.

| Serum | Intra-assay variation, n = 20 | | Inter-assay variation, n = 4 x 6 | |
|-------|-------------------------------|--------|----------------------------------|--------|
| | Mean value (IU/ml) | CV (%) | Mean value (IU/ml) | CV (%) |
| 1 | 157 | 4.7 | 173 | 4.9 |
| 2 | 318 | 2.8 | 338 | 2.9 |
| 3 | 543 | 2.9 | 544 | 6.5 |
| 4 | 713 | 3.6 | 700 | 9.0 |

Correlation of the ELISA with conventional test systems: 209 SLE sera were investigated with the Anti-dsDNA-NcX ELISA, an Anti-dsDNA RIA and a conventional Anti-dsDNA ELISA. The clinical sensitivity of the Anti-dsDNA-NcX ELISA amounted to 60% and clearly exceeded the sensitivity determined for the Anti-dsDNA RIA (52%) and the Anti-dsDNA ELISA (42%).

| SLE, n = 209 | | Anti-dsDNA-NcX ELISA | |
|------------------|----------|----------------------|----------|
| | | positive | negative |
| Anti-dsDNA RIA | positive | 96 | 12 |
| | negative | 29 | 72 |
| Anti-dsDNA ELISA | positive | 86 | 2 |
| | negative | 39 | 82 |

Reference range: Levels of anti-dsDNA antibodies were investigated in 400 sera from healthy blood donors between 18 and 69 years of age (176 women, 224 men) using the EUROIMMUN ELISA. No differences with respect to age or gender were observed. The mean concentration of antibodies against dsDNA was 6.8 RU/ml (\pm 8.2 RU/ml of standard deviation) and the values ranged from 0.6 to 71.8 RU/ml. With a cut-off of 100 IU/ml none of the blood donors were anti-dsDNA positive.



| n = 400 Blood donors | | | |
|----------------------|------------|------------|------------|
| Percentile | 95. | 98. | 99. |
| Cut-off | 16.9 IU/ml | 36.3 IU/ml | 45.4 IU/ml |

ROC analysis: In an analysis of 209 SLE samples and 760 control sera (controls for specificity calculation plus 165 samples from RA patients, 26 samples from myositis patients and 400 samples from healthy blood donors) the following results were achieved:

| Cut-off | Specificity | Sensitivity |
|-------------|-------------|-------------|
| 50.7 IU/ml | 95% | 71% |
| 73.2 IU/ml | 98% | 65% |
| 110.2 IU/ml | 99% | 58% |

Technical data:

Antigen Nucleosome-complexed (NcX) dsDNA coupled to the solid phase.

Calibration Quantitative, in international units per milliliter (IU/ml).

Calibration serum 1: 800 IU/ml
Calibration serum 2: 100 IU/ml; cut-off
Calibration serum 3: 10 IU/ml

Sample dilution Serum or plasma; 1:201 in sample buffer.

Reagents Ready for use. Exception: wash buffer (10x). Colour-coded solutions, in most cases exchangeable with those in other EUROIMMUN ELISA kits.

Test procedure 30 min / 30 min / 15 min. Room temperature. Fully automatable.

Measurement 450 nm. Reference wavelength between 620 nm and 650 nm.

Test kit format 96 break-off wells. Kit includes all necessary reagents.

Order no. EA 1572-9601 G