ELISA kits available from ADI (see details at the web site)

3100	Human anti-dsDNA IgG ELISA Kit, 96 tests, Quantitative
3105	Human anti-dsDNA IgM ELISA Kit, 96 tests, Quantitative
3110	Human anti-dsDNA IgA ELISA Kit, 96 tests, Quantitative
3115	Human anti-ssDNA IgG ELISA Kit, 96 tests, Quantitative
3205	Human Anti-Nuclear Antibodies (ANA) ELISA Kit, 96 tests, Semi-
3210-SSA	Human anti-SS-A/Ro IgG ELISA Kit, 96 tests, Quantitative
3220-SSB	Human anti-SS-B/La IgG ELISA Kit, 96 tests, Quantitative
3250	Human Anti-thryroid peroxidase ELISA kit, Semi-Quantitative
3300	Human Anti-helicobacter pylori IgG ELISA kit, Semi-Quantitative
3300-100-SMG	Human Anti-Smith antigen (Sm) IgG ELISA kit, 96 tests, Quantitative
3300-110-SRG	Human Anti-Smith antigen/RNP (Sm/RNP) IgG ELISA kit, 96 tests,
3300-120-RNG	Human Anti-RNP (RNP-70) IgG ELISA kit, 96 tests, Quantitative
3300-130-HNG	Human Anti-histones IgG ELISA kit, 96 tests, Quantitative
3300-140-SCG	Human Anti-Scl-70 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-150-JOG	Human Anti-Jo-1 (Scleroderma 70 Kda/DNA-topoisomerase-1) IgG
3300-160-AFG	Human Anti-Alpha Fodrin IgG ELISA kit, 96 tests, Quantitative
3300-170-CLG	Human Anti-Cardiolipin IgG ELISA kit, 96 tests, Quantitative
3300-175-CLM	Human Anti-Cardiolipin IgM ELISA kit, 96 tests, Quantitative
3300-185-CLA	Human Anti-Cardiolipin IgA ELISA kit, 96 tests, Quantitative
3300-190-B2G	Human Anti-Beta2-Glycoprotein 1 IgG ELISA kit, 96 tests, Quantitative
3300-195-B2M	Human Anti-Beta2-Glycoprotein 1 IgM ELISA kit, 96 tests, Quantitative
3300-200-APS	Human Anti-Phospholipid Screen IgG/IgM ELISA kit, 96 tests,
3300-200-B2A	Human Anti-Beta2-Glycoprotein 1 IgA ELISA kit, 96 tests, Quantitative
3300-210-PSS	Human Anti-Phosphotidyl serine IgG/IgM ELISA kit, 96 tests,
3300-215-PIS	Human Anti-Phosphotidyl Inositol IgG/IgM ELISA kit, 96 tests,
3300-220-PAS	Human Anti-Phosphotidic Acid IgG/IgM ELISA kit, 96 tests,
3300-230-APG	Human Anti-Prothrombin IgG/IgM ELISA kit, 96 tests, Quantitative
3300-235-APA	Human Anti-Prothrombin IgA ELISA kit, 96 tests, Quantitative
3300-240-AVA	Human Anti-Annexin V IgG ELISA kit, 96 tests, Quantitative
3300-250-ANG	Human ANCA Screen (Anti-PR3 and Anti-MPO) IgG ELISA kit, 96
3300-255-PRG	Human ANCA (Anti-PR3) IgG ELISA kit, 96 tests, Quantitative
3300-265-MPG	Human ANCA (Anti-MPO) IgG ELISA kit, 96 tests, Quantitative
3300-270-GBG	Human Anti-glomerular basement membrane (GBM) IgG ELISA kit, 96
3300-280-BPG	Human Anti-bactericidal permeability increasing (BPI) protein IgG
3300-290-ELG	Human Anti-Elastase IgG ELISA kit, 96 tests, Quantitative
3300-300-GLG	Human Anti-Gliadin IgG ELISA kit, 96 tests, Quantitative
3300-305-GLM	Human Anti-Gliadin IgM ELISA kit, 96 tests, Quantitative
3300-310-GLA	Human Anti-Gliadin IgA ELISA kit, 96 tests, Quantitative
3300-315-PRG	Human Anti-Parietal cell (alpha and beta subunits of the Parietal Cell (H//K/ATPase) IgG ELISA kit, 96 tests,
3300-320-ASC	Human Anti-ASCA (mannan from Saccharomyces cerevisiae) IgA/IgG
3300-330-ASG	Human Anti-Sperm IgG ELISA kit, 96 tests, Quantitative
3300-340-CCG	Human Anti-Cyclic Citrullinated Peptide (CCP)IgG ELISA kit, 96 tests,
3300-350-TPG	Human Anti-thyroid peroxidase (TPO) IgG ELISA kit, 96 tests,
3300-360-TGG	Human Anti-thyroglobulin (TG) IgG ELISA kit, 96 tests, Quantitative
3310	Human Anti-helicobacter pylori IgM ELISA kit, Semi-Quantitative
3320	Human Anti-helicobacter pylori IgA ELISA kit, Semi-Quantitative
3600-HIG	Human Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3610-MKG	Monkey Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3700-MIG	Mouse Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3710-MIM	Mouse Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
3750-RIG	Rat Anti-Insulin IgG ELISA Kit, 96 tests, Quantitative
3760-RIM	Rat Anti-Insulin IgM ELISA Kit, 96 tests, Quantitative
4000	Mouse Anti-Myelin Oligodendrcyte protein (MOG35-55) Ig's ELISA kit,

Instruction Manual No. M-3105

Anti-dsDNA Antibody (Anti dsDNA IgM)

ELISA KIT Cat. No. 3105

For Quantitative Determination of Anti-ds-DNA IgM In Human Serum

For In Vitro Research Use Only

ALPHA DIAGNOSTIC INTERNATIONAL

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

ELISA KIT #3105, Kit Contents: (reagents for 96 tests)

Components	
Native, double-stranded DNA Coated microwell (8 x 12 strips), Ready-to-use, #3 1 0 6	1 Plate
Anti-DNA-IgM **Std A**, 0 U/ml; 1.5 ml, #3 1 0 7 A	1 vial
Anti-DNA-IgM **Std B**, 12.5 U/ml; 1.5 ml, #3 1 0 7 B	1 vial
Anti-DNA-IgM **Std C**, 25 U/ml; 1.5 ml, #3 1 0 7 C	1 vial
Anti-DNA-IgM **Std D**, 50 U/ml; 1.5 ml, #3 1 0 7 D	1 vial
Anti-DNA-IgM **Std E**, 100 U/ml; 1.5 ml, #3 1 0 7 E	1 vial
Anti-DNA-IgM **Std F**, 200 U/ml; 1.5 ml, #3 1 0 7 F	1 vial
Stds are calibrated against the WHO refs WO/80.	
Anti-DNA IgM **Positive control,** 1.5 ml, #3 1 0 5 P	1 vial
Anti-DNA IgM **Negative control,** 1.5 ml, #3 1 0 5 N	1 vial
Sample Buffer (5X), 20 ml , Dilute stock to a final volume of 100 ml distilled water #3 1 0 8	1 bottle
Anti-hIgM HRP-**Conjugate (light red)**, 15 ml, #3 1 0 9	1 bottle
Wash Buffer (50X); 20 ml, **dilute 1:50 with distilled water, #** W - 5 0	1 bottle
HRP Substrate **Solution**, 15 ml, #T M B - 3 1 0 5	1 bottle
Stop Solution, 15 ml, # S T - 1 0	1 bottle
Complete Instruction Manual	3 1 0 5

ADI's ds-DNA IgM assay, a sandwich ELISA, provides a rapid quantitative measurement of anti-ds-DNA IgM in human serum or plasma.. This test is for in vitro research use only.

INTRODUCTION

The presence of serum antibodies to native ds-DNA is one of the major criterion for systemic lupus erythematosus (SLE). These autoantibodies are rarely found in patients with other rheumatic diseases, and their levels especially of those with complement fixing activity, often correlate with active disease. An increase in anti-dsDNA antibodies > 30 IU/ml in less than 10 weeks in conjunction with a decrease in C4 levels is a reliable indicator of exacerbation of SLE.

Antibodies to DNA can be differentiated into 2 groups:

1. Antibodies that bind only to native dsDNA and
2. Antibodies to ssDNA.

Most antibodies to DNA are directed against the phospage units of DNA. Thus, these autoantibodies also bind to ssDNA. However, in serum of SLE patients anti-ssDNA antibodies are found in with a frequency of up to 87% during acute phases and 43% during inactive phases. SLE-like diseases are caused by some drugs. For differential diagnosis of druge-induced SLE, the determination of anti-ssDNA is valuable diagnostic toll. In drug induced SLE, anti-ssDNA is elevated in 50% of cases. Anti-ssDNA has been reported in mononucleosis, hepatitis and various forms of leukemia.

Interpretation of results

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

	Anti-dsDNA IgM [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 of serum Anti-dsDNA. The values above should be regarded as guidelines only.

Parallelism

Human samples were serially diluted 1:100, 1:200, 1:400, 1:800 and anti-ds-IgM measured in the assay. Recovery was 89-105%

Precision

The kit displayed good precision:
Iinter-assay (5-15 CV%)
Intrassay: 6-9CV%

SPECIFICITY

Serum samples known to be positive for extractable nuclear antibodies (ENA), anti-single stranded (ss)-DNA, anti-rheumatoid factor (RF), anti-toxoplasma gondii IgG, IgM, and anti-cytomegalovirus IgG were tested in anti-DNA ELISA kit were found to be negative, confirming the specificity of this assay. dsDNA coated onto the plate is unable to have any ssDNA so the antibodies detected are specific to dsDNA

Sensitivity
1.0 U/ml.

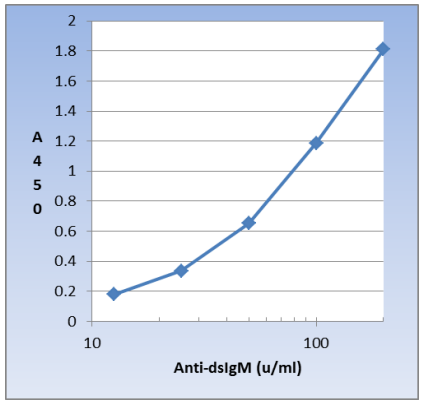
Interference
Hemoglobin up to 1000 mg/dL and lipids (3 g/dL) and bilirubin up to 40 mg/dL or anticoagulant have no interference in the test. However, grossly hemolyzed samples be avoided.

References: Koffer, D. (1974) Annu. Rev. Med. 25: 149-164; Winfield, J.B et all, (1977) SLE. J. Clin. Invest. 59:90-96; Schur, P.H, (1978) N. Engl. J. Med. 278:533; Smeennk R I (1980).J Immunol. Methods 39, 165-180; Szegedi G, (1982) J. Immunol. Methods, 48, 169-175;

WORKSHEET OF TYPICAL ASSAY

Wells	Controls /samples	mean $A_{450\ nm}$	Calculated Concn. (U/ml)*
A1, A2	**Sample Diluent** (Blank)		
B1, B2	**Std A (0 U/ml)**	0.023	
C1, C2	**Std B (12.5 U/ml)**	0.178	
D1, D2	**Std C (25 U/ml)**	0.334	
E1, E2	**Std D (50 U/ml)**	0.652	
F1, F2	**Std E (100 U/ml)**	1.185	
G1, G2	**Std F (200 U/ml)**	1.81	

NOTE: A complete set of positive controls and standards must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values, particularly the cut-off control values for its own sub-population.



A typical Anti-dsDNA IgG Std Curve (do not use this for calculation)

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each duplicate. Plot the concentration of (X) of each standard against the mean Absorbance (Y) on semi-log paper or use 4-parameter-fit with lin-log.
2. Obtain the values of samples from the standard curve.
3. Multiply the sample values only if the samples were diluted more than 1:100 (e.g., if sample diluted by 1:500 then multiply the values by 5).

PRINCIPLE OF THE TEST

Anti-ds-DNA IgM ELISA kit is based on binding of ds-DNA antibody from serum samples to native ds-DNA (recombinant) immobilized on microtiter wells. After a washing step, goat anti-human IgM-HRP conjugate is added. After another washing step, to remove all the unbound enzyme conjugate, chromogenic substrate (TMB) is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of ds-DNA IgM present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of anti-DNA IgM in samples is calculated on the basis of the absorbance of the negative, positive, and, calibrator standards..

MATERIALS AND EQUIPMENT REQUIRED

Adjustable (5-100 µl) and multichannel pipet with disposable plastic tips. Reagent troughs, wash bottle or ELISA plate Washer and Reader.

PRECAUTIONS

This ELISA kit is intended for *in vitro research* use only. The reagents contain thimerosal as preservative; necessary care should be taken when disposing solutions. The negative, positive, and calibrator standards has been prepared from human sera shown to be negative for HBsAg and HIV antibodies. Nevertheless, such tests are unable to prove the complete absence of viruses, therefore, sera should be handled with appropriate precautions.

Applicable **MSDS**, if not already on file, for the following reagents can be obtained from ADI or the web site.

TMB (substrate), H2SO4 (stop solution), and Prolcin-300 (0.1% v/v in standards, sample diluent and HRP-conjugates). All waste material should be properly disinfected before disposal. Avoid contact with the stop solution (1N sulfuric acid).

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture, allowed clot, and serum separated by centrifugation at room temperature. Do not heat inactivate the serum.. If sera can not be immediately assayed , these could be stored at -20^{O}C for up to six months. Avoid repeated freezing and thawing of samples. No preservatives should be added to the serum.

REAGENTS PREPARATION

Wash buffer(50X): Dilute (1:50) with distilled water (20 ml stock to final volume of 1000 ml). Store at 4oC.

Sample Buffer: Dilute 5X stock to a final volume of 100 ml distilled water.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8^{o}C until the expiration date printed on the label. The whole kit stability is usually 12 months from the date of shipping under appropriate conditions. **Do not** contaminate the bottles. Withdraw solutions in a separate clean tube or dispensing trays. Any unused solution should be discarded and not returned to the bottle. Do not use HRP substrate solution if this solution is blue. Do not expose these solutions to strong light.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMP. BEFORE USE).*

Dilute wash buffer (1:50) with distilled water (20 ml stock to final volume of 1000 ml) & Sample Buffer: Dilute 5X stock to a final volume of **100 ml distilled water**.

Dilute serum samples (1:100 with sample buffer; e.g., 10 ul sample in 990 ul buffer). .Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag.

1. Pipet **100 ul** of *Standards, and* positive controls, and *diluted* serum samples into appropriate wells in *duplicate*. Mix gently, cover the plate and incubate for **30 minutes** at 28-30 oC.

2. Aspirate and wash the wells **3 times** with 300 ul of diluted wash buffer. We recommend using an automated ELISA plate washer for better consistency. **Failure to wash the wells properly will lead to high blank values.** If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.

3. Add **100 ul of enzyme conjugate** into each well**.** Mix gently. Cover the plate and incubate for **15 minutes** at room temp.

4. Aspirate and **wash the wells 3 times** as in step3 above.

5. Dispense **100 ul TMB substrate per well**. Mix gently. Cover the plate and incubate for **15 minutes** at room temperature.

6. Stop the reaction by adding **100 ul** of stop solution to all wells. Mix gently. Measure the absorbance at 450 nm using an ELISA reader within 15 min.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed five minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a set of negative & positive standards and calibrator on each plate. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each well the same by adding the reagents in identical sequence.

Quality Control

Positive control/calibrator included in this kit must always be within the range indicated on the vial. Each laboratory must also run other internal controls to monitor assay performance.

LIMITATIONS

For diagnostic purpose, the anti-DNA IgM values should be use as an adjunct to other data available to the physician. A positive test suggests certain diseases, but is not diagnostic and should be confirmed by other clinical findings.