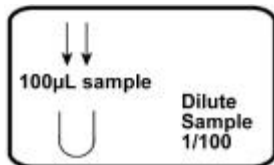
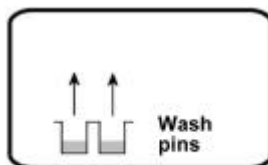


Test Procedure

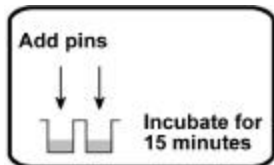
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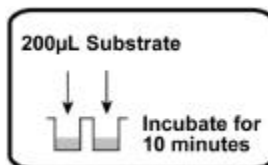
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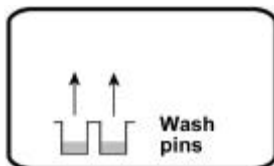
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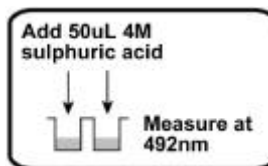
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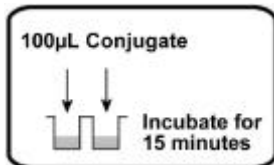
3.



7.



4.

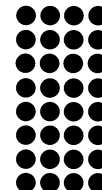


MELISA™

Anti-nDNA Abs

Code: M4196

Instructions for Use



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SCIENCES



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1. Intended Use

The **Anti-native DNA MELISA™** is a sandwich enzyme immunoassay (EIA) for the quantitative detection of anti-native DNA antibodies (anti-nDNA) in human serum or plasma.

The anti-nDNA standards are calibrated against the First International Standard, coded Wo/80 and obtainable from the custodian of WHO: Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, PO Box 9190, 1006 AD Amsterdam, The Netherlands.

2. Background

High levels of auto-antibodies to native DNA (nDNA) are universally regarded as disease markers for systemic lupus erythematosus. In some cases their level is proportional to the clinical disease activity.

Early recognition is vital, coupled with early treatment. In these cases, drug combinations can lower the anti-nDNA level, thereby avoiding long-term risks such as renal or central nervous system involvement.

3. Principle

The anti-nDNA MELISA™ employs a unique antigen-coated micropin technology, which is ideal for the batch-screening of large numbers of samples for anti-nDNA. The method utilises a non-competitive sandwich enzyme immunoassay system.

First Incubation:

MELISA™ pins are provided coated with purified antigen. When the pins are immersed into wells containing standards, controls or diluted sera, any anti-nDNA present will bind to the pin surface. The pins are then removed and washed in wash buffer.

Second Incubation:

The pins are then dipped into wells containing anti-human-IgG/IgM-peroxidase conjugate, which will bind to any captured antibodies. Unbound conjugate is removed by washing in wash buffer.

Third Incubation:

The pins are then dipped into wells containing a colourless substrate. The intensity of the yellow colour formed is proportional to the concentration of anti-nDNA bound in the first incubation. The reaction is stopped with a low pH solution.

4. Kit Contents

7 vials anti-nDNA standards 0.5mL (ready to use) each:

Standard	Anti-nDNA IU/mL
1	0
2	25
3	50
4	100
5	200
6	400
7	800

Nossent, J.C. *et al* (1989) Low avidity antibodies to double stranded DNA in systemic lupus erythematosus: a longitudinal study of their clinical significance. *Ann Rheum Diseases*, **48**, 677-682.

Nossent, J.C. *et al* (1989) Low avidity antibodies to dsDNA as a diagnostic tool. *Ann Rheum Diseases*, **48**, 748-752.

Rubin, R.L. *et al* (1983) An improved ELISA for anti-native DNA by elimination of interference by anti-histone antibodies. *J Immunol. Methods*, **63**, 359-366.

Swaak, A.J.G. *et al* (1982) Prognostic value of anti-dsDNA in SLE. *Ann.Rheum.Diseases*, **41**, 388-395

Tzioufas,A.G. *et al* (1990) Determination of anti-dsDNA antibodies by three different methods: comparison of sensitivity, specificity and correlation with lupus activity index (LAI). *Clin.Rheumatology*, **9/2**, 186-192.

<i>Inter assay</i> (n=3)	Mean (IU/mL)	CV %
Sample 1	473	3.5
Sample 2	274	2.6
Sample 3	89	3.3

3. Minimum detectable concentration

The minimum detectable concentration or sensitivity, defined as the concentration equal to 2 standard deviations from the mean of the sample diluent, was found to be less than 0.8 IU/mL anti-nDNA (n=47).

14. Bibliography

Bardana, E.J. *et al*, (1975) The prognostic and therapeutic implications of DNA:anti-DNA immune complexes in systemic lupus erythematosus (SLE). *Am.J.Med.*, **59**, 515-522.

Feltkamp, T.E.W. *et al* (1988) The first international standard for antibodies to double stranded DNA. *Ann Rheum Diseases*, **47** 740-746.

Hughes, G.R.V. *et al* (1971) Anti-DNA activity in SLE: A diagnostic and therapeutic guide. *Ann Rheum Diseases*, **30** 259-264.

Isenberg, D.A. Shoenfeld, Y. and Schwartz, R.S. (1984) Multiple serologic reactions and their relationship to clinical activity in systemic lupus erythematosus. *Arthritis Rheum*, **27**, 132-138.

- 1 vial wash buffer concentrate, 20 mL
- 1 vial sample diluent concentrate, 20 mL
- 1 vial conjugate concentrate (anti-IgG/IgM-HRP)
- 1 vial conjugate diluent, 15mL
- 1 vial substrate (lyophilised o-phenylenediamine-HCL, toxic)
- 1 vial substrate buffer, 22mL (contains 6% hydrogen peroxide)
- 1 vial 4M sulphuric acid, 7mL (corrosive)
- 1 foil sachet, containing 1 set of antigen-coated micropins and 1 flat-well plate
- 1 vial Negative Control, 1mL (lyophilised)
- 1 vial Mid Positive Control, 1mL (lyophilised)
- 1 vial High Positive Control, 1mL (lyophilised)
- 2 round-well plates
- 2 wash trays
- 1 instruction leaflet
- 1 QC certificate

5. Storage

The kit should be stored refrigerated at 2°C to 8°C. Do not use the reagents beyond their expiry date. Do not freeze.

The reconstituted substrate should be used within 30 minutes of preparation, and all other prepared reagents within 4 hours.

Keep all reagents away from direct sunlight.

6. Safety Precautions

For *in-vitro* diagnostic use only.

For Professional use only.

Safety data sheets are available on request.

4M Sulphuric acid is corrosive and can cause severe burns. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Never add water to this product.

In case of accident, or if you feel unwell, seek medical advice immediately (show the label where possible).

The **substrate** solution (o-phenylenediamine-HCL) is toxic by inhalation, in contact with skin and if swallowed. It may cause sensitisation by skin contact. After contact with skin, wash immediately with plenty of water. If you feel unwell, seek medical advice.

The **standards** and **controls** contain human source material. Although such materials have been found negative when tested for HIV-1 and HIV-2 antibodies, HCV and hepatitis B surface antigen, no test can guarantee their absence. Therefore, the standards and controls should be handled using the same safety precautions employed when handling any potentially infectious material.

Used standards, controls, samples, pipette tips and pins/plates should be handled as clinical waste and incinerated or disposed of in accordance with local rules. Other reagents should be diluted and flushed down the drain. It is recommended that gloves are worn when handling such items.

7. Sample Handling

The Anti-nDNA MELISA™ may be performed on human serum or plasma samples.

Any sample giving values above the standard range should be diluted and retested.

12. Quality Control

Good laboratory practice requires that quality control specimens be included in every run to check on assay performance. Quality Control material for this assay is included in the kit. The control ranges are provided on the QC Certificate. If any of the control values fall outside the quoted range, the results are invalid and the assay should be repeated.

13. Performance Characteristics

1. Expected values:

Anti-nDNA MELISA™ was used to determine the anti-nDNA levels of 100 blood donor plasmas. The mean value + 3 standard deviations (after statistical elimination of 1 outlier) was 47 IU/mL.

It is recommended that a cut-off value of 100 IU/mL be employed when using this kit for screening purposes. Each laboratory, however, should determine its own reference range.

2. Precision:

<i>Intra-assay</i> (n=5)	Mean (IU/mL)	CV %
Sample 1	493	5.2
Sample 2	287	3.8
Sample 3	90	2.5

6. After incubation, transfer the pins to the first wash tray and gently agitate by sideways and up and down movements for at least 10 seconds. Flick off the excess wash buffer or gently drain on absorbent paper. Repeat in the second wash tray, afterwards ensuring that the pins are free of wash buffer and bubbles.
7. Transfer the pins to the conjugate plate and incubate for 15 minutes. During the incubation period, prepare the substrate plate by dispensing 200 μL reconstituted substrate into each well of the **flat-well plate**. Also, prepare for the final pin washing stage by replenishing the wash trays with 60 mL wash buffer.
8. After incubation, wash the pins as in step 6.
9. Transfer the pins to the substrate plate and incubate for 10 minutes.
10. After incubation, carefully remove the pins from the plate and discard them. Add 50 μL of **4M sulphuric acid** to each well. The developed colour will change from yellow to golden brown.
11. Measure the absorbance at 492nm on a 96-well microplate reader.

11. Calculation of Results

For each assay, prepare a calibration curve by plotting mean absorbance against standard concentration on linear graph paper, and interpolate unknowns. Alternatively, use a suitable computerised curve-fit program.

Samples should be assayed within 24 hours of collection or stored frozen at -15°C or colder. Repeated freeze-thawing is not advisable.

Do not heat treat samples prior to assay.

8. Additional Reagents and Equipment

Deionised or freshly distilled water.

Precision micropipettes and disposable tips to deliver 10 to 1000 μL .

Multichannel micropipette or repeating dispenser to deliver 50 μL , 100 μL and 200 μL .

100 mL and 500 mL measuring cylinders for reagent preparation.

96-well microplate reader with a 492nm filter.

Software package (optional).

9. Procedural Precautions

Allow all reagents, patient samples and control sera to equilibrate to ambient temperature (18 to 25°C) before use. Once equilibrated, use within 24 hours. All steps must be performed at 18 to 25°C .

Do not mix reagents from different batches or use beyond their stated expiry.

Keep all reagents away from direct sunlight during storage and incubation.

Always change pipette tips between different standards, samples or control sera to prevent sample carryover.

Never allow the same pipette tip to be used with different reagents. Special care is needed to prevent contamination of the substrate by the conjugate.

The reconstituted substrate should be colourless. Any discoloration indicates that the reagent has been contaminated and must be discarded.

Reconstituted kit controls can be stored at 2 - 8°C, but must be used within 24 hours.

The pin washing procedure is critical for the successful performance of the test, especially between the conjugate and substrate incubations (i.e. the second and third incubations).

Do not use the kit beyond the expiry date given on the box.

Reagents must not be re-used.

10. Assay Procedure

1. Prepare the following reagents:
wash buffer - dilute contents of the **wash buffer concentrate** vial to 300 mL with deionised or freshly distilled water;
sample diluent - dilute the contents of the **sample diluent concentrate** vial to 100 mL with deionised or freshly distilled water;

conjugate - combine the contents of the **conjugate concentrate** vial with the **conjugate diluent**;

reconstituted substrate - carefully add the contents of the **substrate buffer** vial to the vial containing the lyophilised **substrate**. Mix well and leave for at least 10 minutes. Ensure the reconstituted substrate is colourless;

reconstituted kit controls – carefully pipette 1mL deionised /distilled water into each vial and leave to stand for 5 minutes. Gently mix. Once reconstituted they are ready to use.

2. Dilute patient samples by 1/100 using the sample diluent e.g. 10 µL sample added to 990 µL diluent. Standards and Controls DO NOT require dilution.
3. Prepare the sample plate by dispensing 100 µL of each **standard**, diluted patient sample or control serum into appropriate wells of a **round-well plate**. It is recommended that samples be tested in duplicate.
4. Remove the antigen-coated pins from the foil sachet, transfer to the sample plate and incubate for 15 minutes. N.B. Do not pre-wash the pins.
During the incubation period, prepare the conjugate plate by dispensing 100 µL conjugate into each well of the second **round-well plate**, and prepare for the pin washing stage by pouring approximately 60 mL of wash buffer into each of the two **wash trays**, so that it fills the central reservoir.