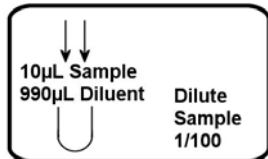
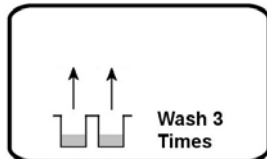


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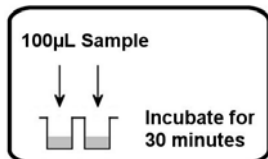
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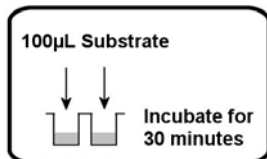
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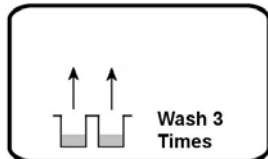
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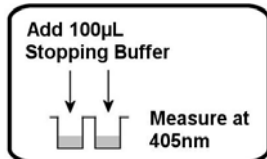
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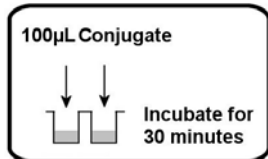
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7.



4.



AUTOZYME™ nDNA

Anti-nDNA antibodies

Code: Z4196

Instructions for Use



Cambridge Life Sciences Ltd
14 St. Thomas' Place
Cambridgeshire Business Park
Ely, Cambs. CB7 4EX UK

Tel: +44(0)1353 645200 Fax: +44(0)1353 645250
email: sales@clsdiagnostics.com
www.clsdiagnostics.com

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Kit Contents Symbols

CAL	Calibrators
CONTROL -	Negative Control
CONTROL +	Positive Control
BUF WASH	Wash Buffer
DIL SPE	Sample Diluent
CONJ	Conjugate solution
SUB	Substrate solution
STOP	Stop Solution
SORB	Solid Phase – Antigen Coated Wells

1. Intended Use

AUTOZYME™ nDNA is an enzyme immunoassay (EIA) for the screening and detection of autoantibodies against native deoxyribonucleic acid (nDNA). The assay is designed to be performed either qualitatively or quantitatively and is used as an aid in the diagnosis and management of Systemic Lupus Erythematosus (SLE).

The calibrator values are traceable to the following international reference preparation: Wo/80.

AUTOZYME™ nDNA has been specifically designed with automation in mind and can be adapted to automated immunoassay systems.

2. Background

Antibodies directed against deoxyribonucleic acid (DNA) were first detected in the serum of patients with SLE in the late 1950s. The presence of anti-nDNA autoantibodies is one of the four highly specific serological markers included in the 1982 American College of Rheumatology (ACR) revised criteria for the classification of SLE. The detection of elevated levels of anti-nDNA antibodies and decreased serum levels of complement component C3 have been found to be 100% specific for SLE.

Antibodies against native (double stranded) DNA (nDNA) are found almost exclusively in patients with SLE, while antibodies against denatured (single stranded) DNA (ssDNA) can also be detected in patients with SLE as well as in patients with many other diseases, including rheumatic diseases and chronic infections.

The serum level of anti-nDNA antibodies in patients with SLE correlates significantly with the level of disease activity, particularly when there is renal involvement. Therefore, the test for anti-nDNA antibodies is useful for monitoring disease activity and the progress of therapy in patients with SLE.

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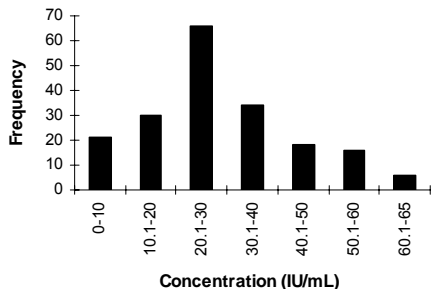
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13. Safety Precautions

For *in vitro* diagnostic use only.
For Professional Use only.

The **substrate** contains ABTS™ which is harmful if swallowed in copious amounts and may cause skin irritation if exposed for prolonged periods. In case of skin contact, wash with soap and water. Flush eyes with copious amounts of water.

The **calibrators and controls** contain human source material. Although 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 calibrators should be handled using the same safety precautions employed when handling any potentially infectious material.

Used calibrators, controls, samples, pipette tips and 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.

ABTS™ (2, 2'-azino-bis (3-ethylbenzothiazoline-6 sulphonic) acid) is a trademark of Roche Diagnostics.

Safety data sheets are available on request.

A variety of tests are commercially available, which incorporate different technologies for the detection of anti-nDNA antibodies, including solution phase assays that use radiolabelled DNA and indirect immunofluorescent assays in which anti-nDNA antibodies bind to the DNA of the hemoflagellate *Crithidia luciliae*. Most of these techniques have inherent limitations. The radiolabelling process of the solution phase assays can denature segments of the DNA, reducing the specificity of these assays for anti-nDNA antibodies. The immunofluorescent *Crithidia* staining assay is labour intensive and requires the subjective interpretation of the staining pattern.

AUTOZYME™ nDNA kit provides rapid objective results and is specific for anti-nDNA antibodies. The antigen-coated wells are specially treated so that no ssDNA is present, even over long-term storage. Results are reported in IU/mL, units traceable to the World Health Organisation standard Wo/80.

3. Principle

AUTOZYME™ nDNA employs a unique antigen-coated microwell technology, which is ideal for the batch screening of large or small numbers of samples for anti-nDNA antibodies.

First Incubation

AUTOZYME™ nDNA wells are provided coated with highly pure native DNA antigen (calf thymus which has been S₁ nuclease treated to remove any residual ssDNA). When calibrators, controls or diluted sera are added any anti-nDNA antibodies present will bind to the well surface. The wells are then washed with buffer to remove any non-specific antibody.

Second Incubation

Goat anti-human IgG/IgM Horseradish peroxidase-conjugated antibodies are added to the well, which will bind to the captured antibody. The wells are then washed with buffer to remove any unbound conjugate.

Third Incubation

A pale green substrate is then added to the wells. The intensity of the green colour formed is proportional to the concentration of anti-nDNA antibodies bound in the first incubation. The reaction is stopped with a low pH solution.

4. Kit Contents

5 vials calibrators (1.5mL ready to use)

Calibrator	Anti-nDNA (IU/mL)
1	0
2	75
3	150
4	300
5	600

1 vial wash buffer concentrate (PBS/Tween), 33 mL (x30)

1 vial sample diluent (BSA/PBS), 100 mL

1 vial conjugate (anti-human-IgG/IgM-HRP), 15 mL

1 vial substrate (ABTS), 15 mL

1 vial stopping buffer (oxalic acid), 15 mL

1 foil sachet containing 1 set of antigen-coated microwells

1 vial Negative Control (1.5 mL ready-to-use)

1 vial Positive Control (1.5 mL ready-to-use)

1 instruction leaflet

1 QC certificate

5. Storage

The kit should be stored at 2 - 8°C. Do not use the reagents beyond their expiry date. Do not freeze. Keep all reagents away from direct sunlight.

12. Performance Data

a. Precision data:

Intra-assay (n=20)	IU/mL	CV%
Sample 1	52.1	16.5
Sample 2	160.0	14.5
Sample 3	132.8	16.1

Inter-assay (n = 6)	IU/mL	CV%
Sample 1	56.1	10.8
Sample 2	201.6	9.3
Sample 3	170.6	17.4

b. Minimum detectable concentration:

The minimum detectable concentration, defined as the concentration equal to 2 standard deviations from the mean of the sample diluent, was found to be 2.5 IU/mL.

c. Reference values:

AUTOZYME™ nDNA was used to determine the anti-nDNA antibody levels of 100 serum samples measured in duplicate from normal blood donors with no apparent abnormalities. The data was evaluated and the following ranges obtained:

Anti-nDNA antibodies:

Normal range ≤ 73.3 IU/mL

Positive > 73.3 IU/mL

10. Calculation of Results

Quantitative Protocol

For each assay, prepare a calibration curve by plotting mean absorbance against calibrator concentration on linear graph paper, and interpolate unknowns. Alternatively, use a computerised curve-fit program. Any sample giving values above the calibrator range should be further diluted and retested.

Qualitative Protocol

A qualitative result using the 75 IU/mL calibrator (calibrator 2) is to be used for screening purposes only.

A ratio is determined by the following calculation:

Mean absorbance of sample
Mean absorbance of calibrator 2

Based on 100 serum samples measured in duplicate from normal blood donors with no apparent abnormalities, the following ranges were achieved:

Ratio \geq 1.0: Positive
Ratio $<$ 1.0: Negative

Positive samples may be accurately quantified using the quantitative protocol.

11. Quality Control

Good laboratory practice requires that quality control samples be included in every run to check on assay performance. The kit control ranges are provided on the certificate of analysis.

If either control value falls outside the quoted range, the results are invalid and the assay should be repeated.

6. Sample Handling

The assay may be performed on human serum samples. 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.

7. Additional Reagents and Equipment Required

Deionised or freshly distilled water.
Precision micropipettes to deliver 10 - 1000 μL .
Multichannel micropipette or repeating dispenser to deliver 100 μL .
1000 mL measuring cylinder for reagent preparation.
Automated plate washer (optional).
96-well microplate reader with 405nm filter.
Software package (optional).

8. Procedural Precautions

Numbering of each strip is advised prior to commencing the assay.

Allow all reagents to equilibrate to room temperature ($18 - 25^{\circ}\text{C}$) before use for a minimum of two hours.

Avoid the use of icteric, lipaemic or grossly haemolysed samples.

Always change tips between different calibrators, 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 substrate should be pale green. Any green colouration (absorbance >0.200) indicates substrate contamination and the substrate should be discarded. The well washing procedure is critical for the successful performance of the test, especially

between conjugate and substrate incubations (i.e. the second and third incubations).

Do not use the kit beyond the expiry date given on the label. Unused reagents are stable at 2 – 8°C for up to 28 days after first opening the container. However, multiple re-use could increase the risk of reagent contamination.

9. Assay Procedure

Quantitative Protocol

1. Prepare the **wash buffer** as follows: dilute contents of the wash buffer concentrate (x30) vial to 1000 mL with deionised water.
2. Dilute patient samples 1/100 using the sample diluent e.g. 10 µL sample added to 990 µL diluent. The **calibrators and kit controls** do not require dilution.
3. Remove the antigen-coated microwells from the resealable sachet. Reseal any unrequired wells in the resealable sachet, along with the desiccant sachet.
4. Dispense 100 µL of each **calibrator, kit control** or **diluted patient sample** into the appropriate wells. Incubate for 30 minutes at room temperature (18°C to 25°C). It is recommended that samples are tested in duplicate.
5. Gripping the frame on the long sides to retain the strips, flick out the contents of the wells. Using the diluted wash buffer, wash the wells three times either with an automated plate washer set to at least 300 µL per well, or by adding 300 µL to each well and flicking out, gripping the frame on the long sides to retain the strips. Alternatively use a wash bottle. Blot the wells on absorbent material to remove any residual liquid.
6. Add 100 µL **conjugate** to each well and incubate for 30 minutes at room temperature.
7. Gripping the frame on the long sides to retain the strips, flick out the contents of the wells.
Wash the wells three times using the same procedure as in step 5.
8. Dispense 100 µL **substrate** into each well, ensuring that it is initially pale green, and incubate for 30 minutes.

9. Stop the reaction by adding 100 µL of **stopping buffer**.
10. Measure the absorbance at 405nm on a 96 well microplate reader.

Qualitative Protocol

1. Prepare the **wash buffer** as follows: dilute contents of the wash buffer concentrate (x30) vial to 1000 mL with deionised water.
2. Dilute patient samples 1/100 using the sample diluent e.g. 10 µL sample added to 990 µL diluent. The **75 IU/mL calibrator (calibrator 2) and kit controls** do not require dilution.
3. Remove the antigen-coated microwells from the resealable sachet. Reseal any unrequired wells in the resealable sachet, along with the desiccant sachet.
4. Dispense 100 µL of **75 IU/mL calibrator (calibrator 2), kit control** or **diluted patient sample** into the appropriate wells. Incubate for 30 minutes at room temperature (18°C to 25°C). It is recommended that samples are tested in duplicate.
5. Gripping the frame on the long sides to retain the strips, flick out the contents of the wells. Using the diluted wash buffer, wash the wells three times either with an automated plate washer set to at least 300 µL per well, or by adding 300 µL to each well and flicking out over a sink, gripping the frame on the long sides to retain the strips. Alternatively use a wash bottle. Blot the wells on absorbent material to remove any residual liquid.
6. Add 100 µL **conjugate** to each well and incubate for 30 minutes at room temperature.
7. Gripping the frame on the long sides to retain the strips, flick out the contents of the wells.
Wash the wells three times using the same procedure as in step 5.
8. Dispense 100 µL **substrate** into each well, ensuring that it is initially pale green, and incubate for 30 minutes.
9. Stop the reaction by adding 100 µL of **stopping buffer**.
10. Measure the absorbance at 405nm on a 96 well microplate reader.