NOVA Lite® dsDNA Crithidia luciliae Kits/Substrate Slides
For In Vitro Diagnostic Use

Product Code: 708200, 708205
508200.10, 508205.20, 508205.80

CLIA Complexity: High

Intended Use
NOVA Lite® dsDNA Crithidia luciliae is an indirect immunofluorescent assay for the screening and semi-quantitative determination of anti-double stranded DNA (dsDNA) in human serum. The presence of anti-double stranded DNA can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of systemic lupus erythematosus (SLE).

Summary and Explanation of the test
The NOVA Lite® dsDNA Crithidia luciliae test is an indirect immunofluorescent antibody test employing the hemoflagellate, Crithidia luciliae, as a substrate. This single-cell organism possesses a giant mitochondrion containing a highly condensed mass of circular dsDNA.1 This mass of dsDNA, known as the kinetoplast, appears to be free of histones or other mammalian nuclear antigens.1,2 It serves as a sensitive and specific substrate for detecting autoantibodies to dsDNA. Autoantibodies to dsDNA occur almost exclusively in patients with systemic lupus erythematosus (SLE) and as such are considered marker antibodies. Autoantibodies to dsDNA have been included in the 1982 Revised Criteria for the Classification of Systemic Lupus Erythematosus by an Arthritis and Rheumatism Association sub-committee.3 While the commonly used Anti-nuclear Antibody (ANA) test is a sensitive screening test for SLE and other connective tissue diseases, it is by no means specific for SLE. Because of this, all positive ANA samples should be tested for specific antibodies to dsDNA. The presence of antibodies to dsDNA strongly indicates SLE; however, absence of these antibodies does not rule out SLE in all cases.

A variety of methods have been employed over the years to detect antibodies to dsDNA. These methods include complement fixation4, passive agglutination5 and RIA.6-8 The main advantage of a C. luciliae based dsDNA test is its specificity, due to the nature of the tightly coiled mass of circular dsDNA in the kinetoplast.9-11 This characteristic is vitally important for a marker antibody test.

Principles of the Procedure
In the indirect immunofluorescence technique, samples are incubated with antigen substrate and unreacted antibodies are washed off. The substrate is incubated with specific fluorescein labeled conjugate and then unbound reagent is washed off. When viewed through a fluorescence microscope, autoantibody positive samples will exhibit an apple green fluorescence corresponding to areas of the cell or nuclei where autoantibody has bound.

Reagents
1. Crithidia luciliae Slides (dsDNA), 6 wells or 12 well/slide, with desiccant

Kits Only
2. Anti-Human IgG Conjugate (Goat) without Evan’s Blue, fluorescein labeled in buffer containing 0.09% sodium azide
3. dsDNA Positive, 1 vial of buffer containing 0.09% sodium azide and human serum antibodies to dsDNA, prediluted
4. IFA System Negative Control, 1 vial of buffer containing 0.09% sodium azide and no human serum antibodies to dsDNA, prediluted
5. PBS II Concentrate (40x)
6. Mounting Medium, 0.09% sodium azide
7. Coverslips

Warnings
1. All human source material used in the preparation of controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method, however, can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the dsDNA Positive and IFA System Negative Control should be handled in the same manner as potentially infectious material.12
2. Sodium Azide is used as a preservative. Sodium Azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
3. Use appropriate personal protective equipment while working with the reagents provided.
4. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

**Precautions**

1. This product (Kit) is for *In Vitro* Diagnostic Use.
2. Substitution of components other than those provided in this system may lead to inconsistent results.
3. Incomplete or inefficient washing of IFA wells may cause high background.
4. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
5. A variety of factors influence the assay performance. These include the starting temperature of the reagents, the strength of the microscope bulb used, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
6. Strict adherence to the protocol is recommended.

**Storage Conditions**

1. Store all the slides and kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Diluted PBS II buffer is stable for 4 weeks at 2-8°C.

**Specimen Collection**

This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results. Microbially contaminated, heat-treated, or specimens containing visible particulates should not be used. Grossly hemolyzed or lipemic serum specimens should be avoided.

Following collection, the serum should be separated from the clot. NCCLS (CLSI) Document H18-A3 recommends the following storage conditions for samples: 1) Store samples at room temperature no longer than 8 hours. 2) If the assay will not be completed within 8 hours, refrigerate the sample at 2-8°C. 3) If the assay will not be completed within 48 hours, or for shipment of the sample, freeze at -20°C or lower. Frozen specimens must be mixed well after thawing and prior to testing.

**Procedure**

**Materials provided (kits)**

**708200**

10 6-well dsDNA *Crithidia luciliae* Substrate Slides  
1 4mL FITC Anti-Human IgG Conjugate  
1 0.5 mL dsDNA Positive  
1 0.5mL IFA System Negative Control  
1 25mL PBS II Concentrate (40x)  
1 7mL Mounting Medium  
1 10 Coverslips  

**708205**

20 12-well dsDNA *Crithidia luciliae* Substrate Slides  
1 15mL FITC Anti-Human IgG Conjugate  
1 0.5 mL dsDNA Positive  
1 0.5mL IFA System Negative Control  
2 25mL PBS II Concentrate (40x)  
1 7mL Mounting Medium  
1 20 Coverslips  

**Materials provided (slides)**

**508200.10** 10 x dsDNA *Crithidia luciliae* Substrate slides (6 well)  
**508205.20** 20 x dsDNA *Crithidia luciliae* Substrate slides (12 well)  
**508205.80** 80 x dsDNA *Crithidia luciliae* Substrate slides (12 well)
**Additional Materials Required But Not Provided**
- Micropipets to deliver 15-1000µL volume
- Distilled or deionized water
- Squeeze bottles or Pasteur pipets
- Moist chamber
- 1L container (for diluting PBS II)
- Coplin jar
- Fluorescence microscope with 495nm exciter and 515nm barrier filter

**Method**

**Before you start**
1. Bring all reagents and samples to room temperature (20-26°C) and mix well.
2. **Dilute PBS II Concentrate**: IMPORTANT: Dilute the PBS II Concentrate 1:40 by adding the contents of the PBS II Concentrate bottle to 975mL of distilled or deionized water and mix thoroughly. The PBS II buffer is used for diluting patient samples and as a wash buffer. The diluted buffer can be stored for up to 4 weeks at 2-8°C.
3. **Dilute Patient Samples**:
   a. Initial Screening: Dilute patient samples 1:10 with diluted PBS II buffer (i.e., add 100µL of serum to 900µL of PBS II buffer).
   b. Titration: Make serial 2-fold dilutions from the initial screening dilution for all positive samples with diluted PBS II buffer (i.e. 1:20, 1:40,... 1:640).

**Assay procedure**
1. **Prepare Substrate Slides**: Allow the substrate slide to reach room temperature prior to removal from its pouch. Label it with pencil and place it in a suitable moist chamber. Add 1 drop (20-25µL) of the undiluted positive and the negative control to wells 1 and 2 respectively. Add 1 drop (20-25µL) of diluted patient sample to the remaining wells.
2. **Slide Incubation**: Incubate the slide for 30 ± 5 minutes in a moist chamber (a dampened paper towel placed flat on the bottom of a closed plastic or glass container) will maintain the proper humidity conditions. Do not allow the substrate to dry out during the assay procedure.
3. **Wash Slides**: After incubation, use a plastic squeeze bottle or pipet to gently wash off the serum with diluted PBS II buffer. Orient the slide and stream of PBS II buffer so as to minimize wash-overflow of samples between wells. Avoid directing the stream directly onto the wells to prevent substrate damage. If desired, place the slides in a Coplin jar of diluted PBS II buffer for up to 5 minutes.
4. **Addition of Fluorescent Conjugate**: Shake off the excess PBS II buffer. Place the slide back in the moist chamber and immediately cover each well with a drop of fluorescent conjugate. Incubate the slides for an additional 30 ± 5 minutes.
5. **Wash Slides**: Repeat Step 3.
6. **Coverslip**: Coverslip procedures vary from lab to lab; however, the following procedure is recommended:
   a. Place a coverslip on a paper towel.
   b. Apply mounting medium in a continuous line to the bottom edge of the coverslip.
   c. Shake off the excess PBS II buffer and touch the lower edge of the slide to the edge of the coverslip. Gently lower the slide onto the coverslip in such a way that the mounting medium flows to the top edge of the slide without air bubble formation or entrapment.

**Quality Control**

dsDNA Positive and IFA System Negative Control should be run on every slide to insure that all reagents and procedures perform properly. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at ≤-70°C. In order for the test results to be considered valid, all of the criteria listed below must be met. If any of these are not met, the test results should be considered invalid and the assay repeated.
1. The undiluted dsDNA Positive must be ≥ 2+.
2. The IFA System Negative Control must be negative.
Interpretation of Results

**Negative Reaction.** A sample is considered negative if the specific kinetoplast staining is less than the negative control. Staining of other structures such as the basal body, the flagellum or the nucleus without concomitant staining of the kinetoplast should be considered negative, for dsDNA reactivity.

**Positive Reaction.** A sample is considered positive if specific kinetoplast staining or kinetoplast plus nuclear staining is observed to be greater than the negative control. All positive samples should be titrated using serial 2-fold dilutions to endpoint. Determine the fluorescence grade or intensity using these criteria:

- 4+ Brilliant apple green fluorescence
- 3+ Bright apple green fluorescence
- 2+ Clearly distinguishable positive fluorescence
- 1+ Lowest specific fluorescence that enables the kinetoplast staining to be clearly differentiated from the background fluorescence

Limitations of the Procedure

1. The user should be aware of the effects of antibody excess and the possibility of obtaining non-dsDNA staining patterns while reading the slides.
2. A variety of external factors influence the test sensitivity including the type of fluorescence microscope used, the bulb strength and age, the magnification used, the filter system and the observer.
3. If a band pass filter is used instead of a 515 barrier filter, increased artifactual staining may be observed.
4. Only pencil should be used to label the slides. Use of any other writing material may cause artifactual staining.
5. All coplin jars used for slide washing should be free from all dye residues. Use of coplin jars containing dye residue may cause artifactual staining.
6. Results of this assay should be used in conjunction with clinical findings and other serological tests.
7. The assay performance characteristics have not been established for matrices other than serum.

Slides sold separately are classified as “Analyte specific reagents”. Except as a component of NOVA Lite® dsDNA *Crithidia luciliae* Kit, analytical and performance characteristics are not established.

**Expected Values**

NOVA Lite® dsDNA *Crithidia luciliae* substrate was used to test a variety of connective tissue disease patients as well as 200 random blood donors. The results appear below:

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Number</th>
<th>NOVA Lite® dsDNA <em>Crithidia luciliae</em> Number Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE</td>
<td>105</td>
<td>42</td>
</tr>
<tr>
<td>Drug Induced Lupus</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>Rheumatoid Arthritis</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>Scleroderma</td>
<td>24</td>
<td>0</td>
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<tr>
<td>Dermatomyositis</td>
<td>14</td>
<td>0</td>
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<tr>
<td>Sjogren’s Syndrome</td>
<td>14</td>
<td>0</td>
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<tr>
<td>Normals</td>
<td>200</td>
<td>0</td>
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References