

# QUANTA Lite™ ssDNA ELISA

708525

single stranded DNA ELISA

For *In Vitro* Diagnostic Use

CLIA Complexity: High

## Intended Use

QUANTA Lite™ ssDNA ELISA is a semi-quantitative Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of anti-single stranded DNA (ssDNA) autoantibodies. The INOVA QUANTA Lite™ ssDNA ELISA kit when used in conjunction with the INOVA QUANTA Lite™ dsDNA kit is designed for the semi-quantitative determination of ssDNA autoantibodies in human serum to aid in the diagnosis of systemic lupus erythematosus (SLE) and certain other rheumatic diseases. The test is not definitive by itself but is one parameter in a multicriterion diagnostic process.

## Summary and Explanation of the test

Antinuclear antibodies (ANA) are found in a wide variety of connective tissue diseases and as such serve as a sensitive screening assay.<sup>1</sup> Antibodies to double stranded (dsDNA) occur almost exclusively in patients with SLE and as such are considered a marker antibody for that disease. The presence of antibodies to dsDNA strongly indicates SLE, however, absence of these antibodies does not rule out SLE in all cases.<sup>2</sup>

Autoantibodies to ssDNA occur in several rheumatic diseases and in various types of other diseases.<sup>3,4</sup> The highest incidence and titer of anti-ssDNA is found in patients with SLE.<sup>4</sup> In SLE patients positive for ssDNA autoantibodies, it has been reported that the titer can be used to monitor changes in disease activity and response to drug therapy.<sup>5,6</sup> While ssDNA antibodies are not specific for SLE, they may be the only positive serologic finding in some SLE patients that fail to demonstrate a significant ANA titer.<sup>7</sup> Many of these patients with high titer ssDNA antibodies exhibit photosensitive cutaneous disease. Approximately 25% of discoid lupus patients possess anti-ssDNA and are at risk to develop systemic features.<sup>3</sup> Presence of anti-ssDNA only in severe discoid lupus patients with glomerulonephritis has been demonstrated.<sup>3</sup> Such studies indicate that ssDNA antibody determinations may be an important diagnostic test for the evaluation of discoid lupus patients or in patients suspected of having ANA negative SLE.<sup>3</sup> Antibodies reacting with thermally denatured ssDNA react against the purines and pyrimidines as well as the sugar-phosphate backbone of the DNA molecule.<sup>1,4</sup> From a technical point of view it is still impossible to measure in a single test true ssDNA antibodies (with purine and pyrimidine bases being the sole antigen). The ELISA technique employed by this assay is objective and results can be reported out in a semi-quantitative fashion. The ELISA procedure can be conveniently used to test both large and small numbers of patient samples in profile format along with other assays for assessing rheumatic diseases such as dsDNA, histone and extractable nuclear antibodies (ENA).

## Principles of the Procedure

A highly purified heat denatured calf thymus ssDNA antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve the antigen in its native state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any ssDNA antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay can be evaluated spectrophotometrically by measuring and comparing the color intensity that develops in the patient wells with the color in the control wells.

## Reagents

1. Polystyrene microwell ELISA plate coated with a purified ssDNA antigen (12-1 x 8 wells), with holder in foil package containing desiccants
2. ELISA Negative Control, 1 vial of buffer containing preservative and human serum with no human antibodies to ssDNA, prediluted, 1.2mL
3. ssDNA ELISA Low Positive, 1 vial of buffer containing preservative and human serum antibodies to ssDNA, prediluted, 1.2mL
4. ssDNA ELISA High Positive, 1 vial of buffer containing preservative and human serum antibodies to ssDNA, prediluted, 1.2mL
5. HRP Sample Diluent, 1 vial – colored pink containing Tris-buffered saline, Tween 20, protein stabilizers and preservative, 50mL
6. HRP Wash Concentrate, 1 vial of 40x concentrate - colored red containing Tris-buffered saline and Tween 20, 25mL. Refer to the Methods Section for dilution instructions.

7. HRP IgG Conjugate, (goat), anti-human IgG, 1 vial – colored blue containing buffer, protein stabilizers and preservative, 10mL
8. TMB Chromogen, 1 vial containing stabilizers, 10mL
9. HRP Stop Solution, 0.344M Sulfuric Acid, 1 vial – colorless, 10mL

## Warnings

1. **WARNING:** This product contains a chemical (0.02% chloramphenicol) in the sample diluent, controls, and conjugate known to the State of California to cause cancer.
2. 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 ssDNA ELISA Low Positive, ssDNA ELISA High Positive and ELISA Negative Control should be handled in the same manner as potentially infectious material.<sup>8</sup>
3. 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.
4. The HRP conjugate contains a dilute poisonous/corrosive chemical, which may be toxic if ingested in large amounts. To prevent possible chemical burns, avoid contact with skin and eyes.
5. TMB Chromogen contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
6. The HRP Stop Solution consists of a dilute sulfuric acid solution. Avoid exposure to bases, metals, or other compounds, which may react with acids. Sulfuric acid is a poison and corrosive, which may be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
7. Use appropriate personal protective equipment while working with the reagents provided.
8. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

## Precautions

1. This product 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 and insufficient liquid removal from the ELISA well strips will cause poor precision and/or 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 ambient temperature, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and liquid removal from the wells of the ELISA strips, the photometer used to measure the results, 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.
7. Incomplete resealing of the zip-lock pouch containing microwell strips and desiccants will result in antigen degradation and poor precision.
8. Unacceptably low absorbencies may be observed following **two** or more uses from a single bottle of HRP conjugate over a period of time. It is important to follow all recommended HRP conjugate handling procedures to prevent this occurrence.
9. Chemical contamination of the HRP conjugate can result from improper cleaning or rinsing of equipment or instruments. Residues from common laboratory chemicals such as formalin, bleach, ethanol or detergent will cause degradation of the HRP conjugate over time. Thoroughly rinse all equipment or instruments after the use of chemical cleaners/disinfectants.

## Storage Conditions

1. Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. Unused antigen coated microwell strips should be resealed securely in the foil pouch containing desiccants and stored at 2-8°C.
3. Diluted wash buffer is stable for 1 week 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 particulate should not be used. Grossly hemolyzed or lipemic serum or specimens should be avoided.

Following collection, the serum should be separated from the clot. NCCLS Document H18-A2 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 hrs, 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

- 1 ssDNA ELISA microwell plate (12-1 x 8 wells), with holder
- 1 1.2mL prediluted ELISA Negative Control
- 1 1.2mL prediluted ssDNA ELISA Low Positive
- 1 1.2mL prediluted ssDNA ELISA High Positive
- 1 50mL HRP Sample Diluent
- 1 25mL HRP Wash Concentrate, 40x concentrate
- 1 10mL HRP IgG Conjugate, (goat), anti-human IgG
- 1 10mL TMB Chromogen
- 1 10mL HRP Stop Solution, 0.344M Sulfuric Acid

### Additional Materials Required But Not Provided

Micropipets to deliver 5, 100, 200-300 and 500µL

Disposable micropipet tips

Test tubes for patient sample dilutions, 4mL volume

Distilled or deionized water

1L container for diluted HRP Wash Concentrate

Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

## Method

### Before you start

1. Bring all reagents and samples to room temperature (20-26°C) and mix well.
2. Dilute the HRP Wash Concentrate 1:40 by adding the contents of the HRP Wash Concentrate bottle to 975mL of distilled or deionized water. If the entire plate will not be run within this period, a smaller quantity can be prepared by adding 2.0mL of the concentrate to 78mL of distilled or deionized water for every 16 wells that will be used. The diluted buffer is stable for 1 week at 2-8°C.
3. Prepare a 1:101 dilution of each patient sample by adding 5µL of sample to 500µL of HRP Sample Diluent. Diluted samples must be used within 8 hours of preparation. **DO NOT DILUTE** the ssDNA ELISA Low Positive, ssDNA ELISA High Positive or ELISA Negative Control.
4. Determination of the presence or absence of ssDNA using arbitrary units requires two wells for each of the three controls and one or two wells for each patient sample. It is recommended that samples be run in duplicate.

### Assay procedure

1. **ALL REAGENTS MUST BE BROUGHT TO ROOM TEMPERATURE (20-26°C) PRIOR TO BEGINNING THE ASSAY.** Place the required number of microwells/strips in the holder. **Immediately return unused strips to the pouch containing desiccants and seal securely to minimize exposure to water vapor.**

2. Add 100µL of the **prediluted** ssDNA ELISA Low Positive, the ssDNA ELISA High Positive, the ELISA Negative Control and the diluted patient samples to the wells. Cover the wells and incubate for 30 minutes at room temperature on a level surface. The incubation time begins after the last sample addition.
3. Wash step: Thoroughly aspirate the contents of each well. Add 200-300µL of the **diluted** HRP Wash buffer to all wells then aspirate. Repeat this sequence twice more for a total of three washes. Invert the plate and tap it on absorbent material to remove any residual fluid after the last wash. It is important to completely empty each well after each washing step. Maintain the same sequence for the aspiration as was used for the sample addition.
4. Add 100µL of the HRP IgG Conjugate to each well. Conjugate should be removed from the bottles using standard aseptic conditions and good laboratory techniques. Remove only the amount of conjugate from the bottle necessary for the assay. **TO AVOID POTENTIAL MICROBIAL AND/OR CHEMICAL CONTAMINATION, NEVER RETURN UNUSED CONJUGATE TO THE BOTTLE.** Incubate the wells for 30 minutes as in step 2.
5. Wash step: Repeat step 3.
6. Add 100µL of TMB Chromogen to each well and incubate **in the dark** for 30 minutes at room temperature.
7. Add 100µL of HRP Stop Solution to each well. Maintain the same sequence and timing of HRP Stop Solution addition as was used for the TMB Chromogen. Gently tap the plate with a finger to thoroughly mix the wells.
8. Read the absorbance (OD) of each well at 450nm within one hour of stopping the reaction. If bichromatic measurements are desired, 620nm can be used as a reference wavelength.

## Quality Control

1. The ssDNA ELISA Low Positive, the ssDNA ELISA High Positive and the ELISA Negative Control should be run with every batch of samples to ensure that all reagents and procedures perform properly.
2. Note that since the ssDNA ELISA Low Positive, the ssDNA ELISA High Positive and the ELISA Negative Control are prediluted, they do not control for procedural methods associated with dilution of specimens.
3. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing at  $\leq -20^{\circ}\text{C}$ .
4. 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 should be considered invalid and the assay repeated.
  - a. The absorbance of the prediluted ssDNA ELISA High Positive must be greater than the absorbance of the prediluted ssDNA ELISA Low Positive which must be greater than the absorbance of the prediluted ELISA Negative Control.
  - b. The prediluted ssDNA ELISA High Positive must have an absorbance greater than 1.0 while the prediluted ELISA Negative Control absorbance cannot be over 0.2.
  - c. The ssDNA ELISA Low Positive absorbance must be more than twice the ELISA Negative Control or over 0.25.
  - d. The ELISA Negative Control and ssDNA ELISA High Positive are intended to monitor for substantial reagent failure. The ssDNA ELISA High Positive will not ensure precision at the assay cutoff.
  - e. The user should refer to NCCLS Document C24-A for additional guidance on appropriate QC practices.

## Calculation of Results

The average OD for each set of duplicates is first determined. The reactivity for each sample can then be calculated by dividing the average OD of the sample by the average OD of the ssDNA ELISA Low Positive. The result is multiplied by the number of units assigned to the ssDNA ELISA Low Positive found on the label.

$$\text{Sample Value (units)} = \frac{\text{Sample OD}}{\text{ssDNA ELISA Low Positive OD}} \times \text{ssDNA ELISA Low Positive (units)}$$

Reactivity is related to the quantity of antibody present in a non-linear fashion. While increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the reactivity). If a more accurate quantitation of patient antibody is required, serial dilutions of the patient sample should be run and the last dilution to measure positive in the assay should be reported as the patient's antibody titer.

## Interpretation of Results

The ELISA assay is very sensitive to technique and is capable of detecting even small differences in patient populations. The values shown below are suggested values only. Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures. The following information serves as an example to assist in interpreting results.

Since both single stranded (ssDNA) and some double stranded (dsDNA) autoantibodies can react with the ssDNA antigen of this kit, patient samples should also be tested for dsDNA reactivity. These results, in WHO units, should be taken into consideration when evaluating ssDNA levels. By combining the results of the INOVA QUANTA Lite™ dsDNA ELISA assay with those of the INOVA QUANTA Lite™ ssDNA ELISA assay one can draw conclusions as to the existence of ssDNA antibodies by calculating the ratio (ssDNA/dsDNA). A ratio  $\leq 0.5$  indicates an absence of ssDNA antibodies. A ratio  $> 0.5$  indicates the presence of ssDNA antibodies.

*NOTE: Only when the result of the ssDNA Antibody assay is positive and the ratio of ssDNA units to dsDNA units is  $> 0.5$  should the patient sample be considered positive.*

Example 1: Assay 1: dsDNA antibody assay concentration: 120 WHO U/mL  
Assay 2: ssDNA antibody assay concentration: 160 U/mL  
ssDNA antibody ratio (ssDNA/dsDNA):  $160/120 = 1.33 (+)$   
Interpretation: Patient sample is positive for ssDNA antibodies

Example 2: Assay 1: dsDNA antibody assay concentration: 270 WHO U/mL  
Assay 2: ssDNA antibody assay concentration: 130 U/mL  
ssDNA antibody ratio (ssDNA/dsDNA):  $130/270 = 0.48 (-)$   
Interpretation: Patient sample is negative for ssDNA antibodies

Example 3: Assay 1: dsDNA antibody assay concentration: 80 WHO U/mL  
Assay 2: ssDNA antibody assay concentration: 80 U/mL  
ssDNA antibody ratio (ssDNA/dsDNA):  $80/80 = 1.0 (+)$   
Interpretation: Patient sample is positive for ssDNA antibodies

If the result of the ssDNA assay is determined to be positive, then the ssDNA units may be compared to the following clinical categories.

Less than 68.6 U/mL	Negative for ssDNA antibodies
68.6 - 229 U/mL	Moderate positive for ssDNA antibodies
over 229 U/mL	Strong positive for ssDNA antibodies

It may be difficult to determine the ssDNA activity in samples exhibiting high positive values (i.e. greater than 2.0 OD) for both ssDNA and dsDNA. When samples are strongly positive the ratio may be misleading because the ELISA is saturated with patient autoantibody. It is recommended to retest samples strongly positive for both ssDNA and dsDNA at a further 1:4 and 1:20 dilution (i.e. 1:404 and 1:2020) in HRP Sample Diluent in order to determine if the ssDNA activity is at a higher level than the dsDNA. The calculations above must be made on the same dilution of patient sample.

## Limitations of the Procedure

1. Since IgG antibodies are thought to be the more clinically significant, this assay uses an IgG specific conjugate. IgM ssDNA antibodies not measured in this assay may compete with IgG antibodies for available binding sites. If interference from IgM antibodies is suspected, reassay the sample at a higher dilution in HRP Sample Diluent. A sharp increase in the value for the sample indicates the presence of competing IgM antibody. This new value will give a more accurate determination of the amount of ssDNA IgG antibodies in the patient sample.
2. The presence of immune complexes or other immunoglobulin aggregates in the patient sample may cause an increased level of non-specific binding and produce false positives in this assay.
3. Since both single stranded (ssDNA) and some double stranded (dsDNA) autoantibodies can react with the ssDNA antigens of this kit, patient samples should also be tested for dsDNA reactivity, and these results be taken into consideration when evaluating ssDNA levels.
4. A variety of factors influence the assay performance. These include the accuracy and reproducibility of pipetting technique, the photometer used to measure the results, and timing bias during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
5. The QUANTA Lite™ ssDNA ELISA is not a stand-alone assay for ssDNA antibodies. It must be used in conjunction with the INOVA Diagnostics, Inc.'s QUANTA Lite™ dsDNA ELISA in order to measure ssDNA antibodies. Do not use a dsDNA Antibody test from a different manufacturer since the calibration of the INOVA ssDNA ELISA and the dsDNA ELISA have been matched.
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.

## Expected Values

The ability of the QUANTA Lite™ ssDNA ELISA to detect ssDNA antibodies was evaluated by comparison to a commercially available ELISA test. Results of the ELISA test were determined according to the manufacturer's direction insert.

### Normal Range

One hundred and seventeen random normal serum samples were selected and tested by the QUANTA Lite™ ssDNA ELISA kit. Of these samples, seven had 69 or more units (a positive result in this ELISA system). Five of these seven samples were also positive in a commercial reference method for anti-ssDNA antibodies and were therefore eliminated from the normal population. The mean for the sample population was 27, with a standard deviation of 21. The range of the population was 12 to 186. This trial indicated that the average normal sample is 2.0 standard deviations below the cutoff.

### Relative Sensitivity and Specificity

Thirty-two random ANA positive samples were tested on the QUANTA Lite™ ssDNA ELISA test and another commercially available kit (Reference). Eighteen samples were positive and 12 samples negative by both methods. Two samples were positive by the reference method yet negative by INOVA. These two samples tested negative in a different commercially available kit. These results are summarized below.

	INOVA			
	+	-		
Reference	+	18	2	Relative Sensitivity 90%
				Relative Specificity 100%
	-	0	12	Relative Efficiency 94%

To further investigate the specificity of the ssDNA solid phase, a variety of high titered, monospecific autoantibody controls were tested in the QUANTA Lite™ ssDNA ELISA kit. These controls included the high level controls from the following INOVA QUANTA Lite™ ELISA kits: Sm, RNP, SS-A, SS-B, Scl-70, Jo-1, Thyroid Microsomal and Thyroglobulin, Histone and Mitochondria M2. All of the above controls gave negative results in the ssDNA assay.

### ssDNA Reactivity In Various Connective Tissue Diseases

	Number of Patients	% Positive
SLE	43	58
Sjogren's Syndrome	21	62
Scleroderma	15	80
Polymyositis	6	17
Drug Induced Lupus	20	80
Rheumatoid Arthritis	11	45*

\*2 of the 5 positive samples were only borderline positive (69 and 99 units).

Twenty-five of the SLE patients were ssDNA positive. Six of these ssDNA positive SLE patients were equivocal with a sensitive double stranded DNA (dsDNA) ELISA method (INOVA). Thirteen of 21 Sjogren's syndrome patients were ssDNA positive. All 21 patients were positive for SS-A, SS-B or both SS-A and SS-B by ELISA (INOVA). Three of these Sjogren's samples were ssDNA positive yet completely negative by a sensitive dsDNA ELISA method (INOVA). Twelve of fifteen Scl-70 positive scleroderma patients were ssDNA positive and only 1 of the 6 polymyositis patients was positive. All of these polymyositis patients were positive for autoantibodies to Jo-1 by ELISA (INOVA). The one polymyositis patient that was ssDNA positive (112U) was completely negative for antibodies to dsDNA by ELISA (INOVA).

### Precision and Reproducibility

The precision and reproducibility of the assay was measured by running six replicates each of negative, moderate positive, and strong positive samples in four separate assays. The mean of the strong positive was 814 units, the moderate positive was 300 units and the negative was 72 units. The standard deviation and coefficient of variation for each sample are summarized below.

	Negative		Strong Positive		Moderate Positive	
	SD	CV	SD	CV	SD	CV
Overall	4.7	6.6%	36	4.5%	8.0	2.7%
Within Run	4.7	6.5%	35	4.3%	6.8	2.7%
Between Run	4.6	6.3%	24	3.0%	8.7	2.9%

## References

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Manufactured By:

INOVA Diagnostics, Inc.  
9900 Old Grove Road  
San Diego, CA 92131  
United States of America

Authorized Representative in the EU:

Medical Technology Promedt Consulting GmbH  
Altenhofstrasse 80  
D-66386 St. Ingbert, Germany  
Tel.: +49-6894-581020  
Fax.: +49-6894-581021  
[www.mt-procons.com](http://www.mt-procons.com)

Technical Service  
628525USA

888-545-9495  
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