Intended Use
NOVA Lite™ Thyroid is an indirect immunofluorescent assay for the screening and semi-quantitative determination of thyroid microsomal and colloid/thyroglobulin antibodies in human serum. The presence of microsomal and/or colloid antibodies can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of autoimmune thyroid disease.

Summary and Explanation of the test
Circulating thyroid autoantibodies have been widely implicated in the etiology of autoimmune thyroid disease and both thyroglobulin and microsomal antibodies are routinely measured in clinical practice. Serum autoantibodies to thyroid microsomal antigen(s) are commonly found in patients with thyroid autoimmune diseases and their presence correlates well with histological changes in Hashimoto’s thyroiditis. The indirect immunofluorescent test for antibodies to thyroid microsomal antigens is positive in 70-90% of patients with chronic thyroiditis. These antibodies are also found in 64% of patients with primary hypothyroidism, 50% with thyrotoxicosis, 10% with simple goiters and 17% with thyroid tumors. Thyroglobulin autoantibodies are detected, at high titers, mainly in autoimmune thyroiditis and Graves’ disease. By immunofluorescence, serum autoantibodies to thyroglobulin/colloid have been found in 40-70% of patients with chronic thyroiditis and in smaller percentages of patients with thyrotoxicosis and nontoxic goiters. Thyroid autoantibodies have been investigated classically by means of precipitation reactions, latex fixation, hemagglutination and by immunofluorescence. Immunofluorescence has proven to be both sensitive and specific in the detection of thyroid autoantibodies. Unlike commonly used hemagglutination techniques, Immunofluorescence will detect nonprecipitating antibodies and is not adversely influenced by presence of agglutination inhibition factors or heterophile antibody.

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 primate absorbed 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 thyroid tissue where autoantibody has bound.

Reagents
1. Thyroid (primate thyroid), 8 wells/slide, with desiccant
2. Anti-Human IgG Conjugate Primate Absorbed (Goat), fluorescein labeled in buffer containing Evans Blue and 0.09% sodium azide, 7mL
3. Thyroid Positive, 1 vial of buffer containing 0.09% sodium azide and human serum antibodies to thyroid, prediluted, 0.5mL
4. IFA Systems Negative Control, 1 vial of buffer containing 0.09% sodium azide and no human serum antibodies to thyroid, prediluted, 0.5mL
5. PBS Concentrate (40x), sufficient for 1000 mL
6. Mounting Medium, 0.09% sodium azide, 7mL
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 Thyroid Positive and IFA Systems Negative Control should be handled in the same manner as potentially infectious material.
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 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. Over time, the Anti-Human IgG Conjugate Primate Absorbed may change in color due to exposure to light. However, the color change does not affect the assay performance.
7. Strict adherence to the protocol is recommended.

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. Diluted PBS 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. Microbi ally 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 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
10 8-well Thyroid Substrate Slides
1 7mL FITC IgG Conjugate Primate Absorbed
1 0.5 mL Thyroid Positive
1 0.5mL IFA Systems Negative Control
1 25mL PBS Concentrate (40x)
1 7mL Mounting Medium
1 10 Coverslips

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)
Method

Before you start
1. Bring all reagents and samples to room temperature (20 - 26°C) and mix well.
2. **Dilute PBS Concentrate**: IMPORTANT: Dilute the PBS Concentrate 1:40 by adding the contents of the PBS Concentrate bottle to 975mL of distilled or deionized water and mix thoroughly. The PBS 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 buffer (i.e., add 100μL of serum to 900μL of PBS buffer).
   b. Titration: Make serial 2-fold dilutions from the initial screening dilution for all positive samples with diluted PBS buffer (i.e. 1:20, 1:40,... 1:2560).

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 (70 - 90μL) of the undiluted positive and the negative control to wells 1 and 2 respectively. Add 1 drop (70 - 90μL) 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 buffer. Orient the slide and stream of PBS buffer so as to minimize wash-over 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 buffer for up to 5 minutes.
4. **Addition of Fluorescent Conjugate**: Shake off the excess PBS 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 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
Thyroid 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 Thyroid Positive must be ≥ 3+.
2. The IFA System Negative Control must be negative.

Interpretation of Results
**Negative Reaction.** A sample is considered negative if specific staining of thyroid epithelial cell cytoplasm (microsomes) or colloid (thyroglobulin) is equal to or less than the negative control.

**Positive reaction for microsomal antibodies.** This reaction manifests as a fluorescent staining of the cytoplasm of epithelial cells. The nucleus is usually unstained.
Positive reaction for thyroglobulin/colloid antibodies. This reaction manifests as a floccular or "ground glass" staining pattern of the colloid. 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 staining of thyroid epithelial cell cytoplasm (microsomes) or colloid (thyroglobulin) to be clearly differentiated from the background fluorescence

Limitations of the Procedure

1. When two or more autoantibodies are present in the same sample, "interference phenomena" may occur. These are characterized by varying fluorescence patterns with different sample dilutions and inconsistent endpoint titers. The presence of both antibody types should be reported.
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.

Expected Values

NOVA Lite™ Thyroid test was used to evaluate 24 patients with chronic thyroiditis (Hashimoto’s). Fifteen patients with type I diabetes and 67 random blood donors with no reported thyroid abnormalities were also tested. The results appear below:

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<table>
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<th>Patient Group</th>
<th>Number</th>
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<th>Colloid</th>
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<tr>
<td>Chronic Thyroiditis</td>
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<td>13</td>
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<tr>
<td>Type I Diabetes</td>
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References