### Materials Provided with the Kit
- Murine Monoclonal Anti-TSH-coated microtiter wells.
- Set of Reference Standards: 0.0, 0.1, 0.5, 2.5, and 10 μIU/mL, lyophilized.
- Enzyme Conjugate Reagent, 13 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.
- Reagents:
  - Enzyme Conjugate Reagent, 13 ml.
  - TMB Reagent (One-Step), 11 ml.
  - Stop Solution (1N HCl), 11 ml.

### Principle of the Test
The Ultrasensitive TSH ELISA test is based on the principle of a solid phase enzyme-immunosorbent assay. The assay system utilizes a monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase immobilization (on the microtiter wells). A goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 2-hour incubation at room temperature, the wells are washed with water to remove unbound labeled antibodies. A solution of TMB Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of TSH is directly proportional to the color intensity.

### Specimen Collection and Preparation
Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

### Assay Procedure
1. Secure the desired number of coated wells in the holder.
2. Dispense 100μl of standards, specimens, and controls into appropriate wells.
3. Dispense 100μl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix completely.
5. Incubate at room temperature (18-25°C) with shaking at 175 RPM, for 120 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100μl of TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100μl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450nm with a microtiter well reader within 15 minutes.

### Calculation of Results
1. Calculate the mean absorbance value (A<sub>450</sub>) for each set of reference standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in μIU/mL on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
Ultra Sensitive
Thyroid Stimulating Hormone (U-TSH)
Enzyme Immunoassay (EIA)

3. Use the mean absorbance values for each specimen to determine the corresponding concentration of TSH in μIU/ml from the standard curve.

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Example of Standard Curve
Results of a typical standard run with absorbency readings at 450nm shown in the Y axis against TSH concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

<table>
<thead>
<tr>
<th>TSH (μIU/ml)</th>
<th>Absorbance (450nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.009</td>
</tr>
<tr>
<td>0.1</td>
<td>0.050</td>
</tr>
<tr>
<td>0.5</td>
<td>0.218</td>
</tr>
<tr>
<td>2</td>
<td>0.801</td>
</tr>
<tr>
<td>5</td>
<td>1.800</td>
</tr>
<tr>
<td>10</td>
<td>3.191</td>
</tr>
</tbody>
</table>

Grave’s disease or thyroiditis. Differential diagnosis is best achieved by simultaneous determination of TSH and free T-4 levels in serum. The minimum detectable concentration of TSH by this assay is estimated to be 0.05 μIU/ml.

Limitations of the Procedure
1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

References

Expected Values and Sensitivity
The mean TSH values based on 160 random normal adult blood samples, is 1.6 (0.4-7.0) μIU/ml. TSH levels exceeding 10μIU/ml, suggest primary hypothyroidism. Low or undetectable TSH levels may be normal, but may also indicate secondary hypothyroidism (insufficient secretion of TSH or TRH). Low levels may also be due to hyper-secretion of T-3 and T-4 due to Grave’s disease or thyroiditis. Differential diagnosis is best achieved by simultaneous determination of TSH and free T-4 levels in serum. The minimum detectable concentration of TSH by this assay is estimated to be 0.05 μIU/ml.

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References