ab108660

Thyroid Stimulating Hormone (TSH) Human ELISA Kit

Instructions for Use

For the quantitative measurement of Human Thyroid Stimulating Hormone (TSH) concentrations in serum.

This product is for research use only and is not intended for in vitro diagnostic use.

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1. Introduction

ab108660, Thyroid Stimulating Hormone (TSH) Human ELISA Kit is intended for the quantitative determination of thyroid stimulating hormone (TSH) concentration in Human serum.

The determination of serum or plasma levels of thyroid stimulating hormone (TSH) or thyrotropin is recognized as a sensitive method in the diagnosis of primary and secondary hypothyroidism. Thyroid Stimulating Hormone (S-TSH) is secreted by the anterior lobe of the pituitary gland and induces the production and release of thyroxine and triiodothyronine from the thyroid gland. It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta.
2. Assay Summary

*ab108660 is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact Thyroid Stimulating Hormone (TSH) molecule.*

Mouse monoclonal anti-Thyroid Stimulating Hormone (TSH) antibody is used for solid phase immobilization (on the microtiter wells). A Goat anti-Thyroid Stimulating Hormone (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 Thyroid Stimulating Hormone (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 tetramethylbenzidine (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 Thyroid Stimulating Hormone (TSH) is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.
3. Kit Contents

- Antibody Coated Microplate 12 x 8 wells
- Antibody HRP Conjugate Reagent 1 x 13 ml
- Stop Solution 1 x 11 ml
- TMB Reagent 1 x 11 ml
- TSH Standard 0 - 0 µIU/mL (Lyophilized) 1 vial
- TSH Standard 1 - 0.1 µIU/mL (Lyophilized) 1 vial
- TSH Standard 2 - 0.5 µIU/mL (Lyophilized) 1 vial
- TSH Standard 3 - 2.0 µIU/mL (Lyophilized) 1 vial
- TSH Standard 4 - 5 µIU/mL (Lyophilized) 1 vial
- TSH Standard 5 - 10 µIU/mL (Lyophilized) 1 vial

4. Storage and Handling

Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above.
5. Additional Materials Required

- Equipment capable of shaking microplates at 175 RPM.
- Distilled or deionized water
- Precision pipettes: 100 µl and 1.0 ml
- Disposable pipette tips
- Microtiter well reader capable of reading absorbance at 450 nm. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.
- Vortex mixer, or equivalent
- Absorbent paper
- Graph paper

6. Preparation of Reagents

1. All reagents should be allowed to reach room temperature (18-25°C) before use.

2. Reconstitute each lyophilized Standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. The reconstituted Standards will be stable for up to 30 days when stored sealed at 2-8°C.
7. Preparation and Collection of Specimen

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.

8. Assay Method

Assay Procedure:

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl 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 100 µl 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 100 µl 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.

**9. Data Analysis**

1. Calculate the mean absorbance value (OD450) for each set of reference standards, controls and samples.

2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in µIU/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of Thyroid Stimulating Hormone (TSH) in µIU/ml from the standard curve.
A. Typical Data

Results of a typical standard run with absorbency readings at 450nm shown on the Y axis against Thyroid Stimulating Hormone (TSH) concentrations shown on the X axis.

NOTE: This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve in each experiment.

<table>
<thead>
<tr>
<th>Thyroid Stimulating Hormone (TSH) (µIU/ml)</th>
<th>Absorbance (450 nm)</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>
B. Sensitivity

The minimum detectable concentration of Thyroid Stimulating Hormone (TSH) by this assay is estimated to be 0.05 µIU/ml.
10. Limitations

- 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.

- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.

- The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

- 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.
## 11. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4 °C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td>Issue</td>
<td>Recommendation</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
<td></td>
</tr>
<tr>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
<td></td>
</tr>
<tr>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes &amp; ensure accurate pipetting</td>
<td></td>
</tr>
<tr>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)</td>
<td></td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
<td></td>
</tr>
<tr>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
<td></td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
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</tbody>
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Technical Support

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