DIAGNOSTIC AUTOMATION, INC.
21250 Califa Street, Suite 102 and 116, Woodland Hills, CA 91367
Tel: (818) 591-3030 Fax: (818) 591-8383
onestep@rapidtest.com
technicalsupport@rapidtest.com
www.rapidtest.com

IVD See external label 2°C 8°C Σ 96 tests REF 3123-16

ULTRASENSITIVE THYROID STIMULATING HORMONE (u-TSH)

TSH Ultra Sensitive

REF **3123-16**

Enzyme Immunoassay for the Ultra sensitive Quantitative Determination of Thyroid Stimulating Hormone (U-TSH) in Human Serum

Test	TSH Ultra Sensitive ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	ELISA - Indirect; Antibody Coated Plate
Sample	50µl Serum
Sensitivity	0.05µIU/mL
Total Time	~140 min
Shelf Life	12 Months from the manufacturing date

Enzyme Immunoassay for the Ultrasensitive Quantitative Determination of Thyroid Stimulating Hormone (U-TSH) in Human Serum

INTENDED USE

The Diagnostic Automation Ultra Sensitive TSH ELISA is for the quantitative determination of the thyroid stimulating hormone (TSH) concentration in human serum.

INTRODUCTION

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.[i] 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.[ii] It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta.

Although the concentration of TSH in the blood is extremely low, it is essential for the maintenance of normal thyroid function. The release of TSH is regulated by a TSH-releasing hormone (TRH) produced by the hypothalamus. The levels of TSH and TRH are inversely related to the level of thyroid hormone. When there is a high level of thyroid hormone in the blood, less TRH is released by the hypothalamus, so less TSH is secreted by the pituitary. The opposite action will occur when there is decreased thyroid hormone in the blood. This process is known as a negative feedback mechanism and is responsible for maintaining the proper blood levels of these hormones.

TSH and the pituitary glycoproteins: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG), have identical alpha chains. The beta chains are distinct but do contain regions with identical amino acid sequences. These regions of homology can cause considerable cross-reactivity with some polyclonal TSH antisera. The use of a monoclonal antibody in this TSH ELISA test eliminates such cross-reactivity, which could result in falsely elevated TSH values in either menopausal or pregnant females -- a population whose evaluation of thyroid status is clinically significant.

PRINCIPLE OF THE TEST

The Ultra sensitive TSH ELISA test 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 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 H_2O_2/TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 3N HCl changing the color to yellow. The concentration of TSH is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-TSH antibody coated microtiter wells.
- Set of Reference Standards: 0, 0.1, 0.5, 2, 4 and 6µIU/ml.
- Enzyme Conjugate Reagent, 12 ml.
- Wash Buffer Concentrate (50X),15 ml
- TMB Substrate, 12 ml.
- Stop Solution, 12 ml.

Materials required but not provided:

- Precision pipettes: 50µl, 100µl, 200µl, and 1.0 ml.

- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.
- Graph paper.
- Microtiter plate reader.

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.

Storage of Test Kit and Instrumentation

Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2.5 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

Reagent Preparation

- All reagents should be brought to room temperature (18-22°C) before use.
- Dilute 50X washing buffer concentrate 1:50 with distilled water to washing buffer (1X) and mix carefully before use.

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 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 (20 +/-2°C) f*or 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 washing buffer (1X).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB solution into each well. Gently mix for 5 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_{450}) for each set of reference standards, controls and patient samples.
2. We recommend to use a proper software to calculate the results. The best curve fitting used in this assay is 4-parameter regression. If the software is not available, 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.
3. Use the mean absorbance values for each specimen to determine the corresponding concentration of TSH in µIU/ml from the standard curve.

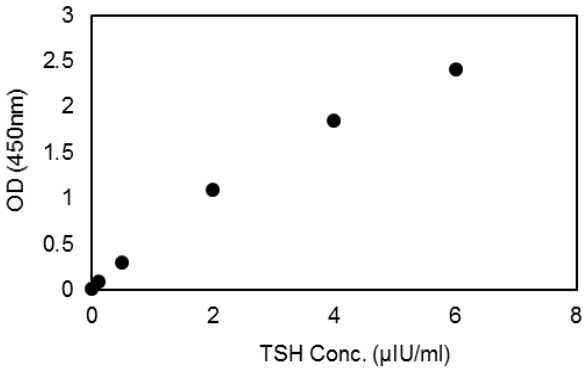
LIMITATIONS OF THE PROCEDURE

1) As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2) Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.
3) The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance. The use of tap water for washing could result in a higher background absorbance.

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.

TSH (μIU/ml)	Absorbance (450nm)
0	0.013
0.1	0.086
0.5	0.294
2	1.096
4	1.843
6	2.414



EXPECTED VALUES AND SENSITIVITY

Based on a study of 139 random normal adult blood samples, normal TSH values and ranges (in μIU/ml) were shown in the following table.

Expected Values		2.5 Percentile (70% Conf Int)	
Low Normal	0.39	Low Range	0.28–0.53
High Normal	6.16	High Range	5.60–6.82

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent on a multiplicity of factors: the quant of the method, the population tested and the precision of the method in the hands of the analyst. Therefore, each laboratory should depend on the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

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 0.05µIU/ml.

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