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

ULTRASENSITIVE THYROID STIMULATING HORMONE (u-TSH)

U-TSH

Cat # 3123-16

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

INTENDED USE

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.[1] 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.[2] It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta.[3]

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. [4,5]

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.[6,7,8]

PRINCIPLE OF THE TEST

The Ultra sensitive TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.[9,10] 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, 5, and 10µ IU/ml.
- Enzyme Conjugate Reagent, 12 ml.
- TMB Substrate, 12 ml.
- Stop Solution, 12 ml.
- Wash Buffer Concentrate(50X),15ml

Materials required but not provided:

- Precision pipettes: 50µl, 100µl, 200µl, and 1.0ml.
- 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 0-3 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 1 volume of Wash Buffer (50x) with 49 voumes of distilled water. For example, Dilute 15 ml of Wash Buffer (50x) into distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

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 (22±-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 30 minutes.

Calculation of Results

1. Calculate the mean absorbance value (A_{450}) 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 µ IU/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.

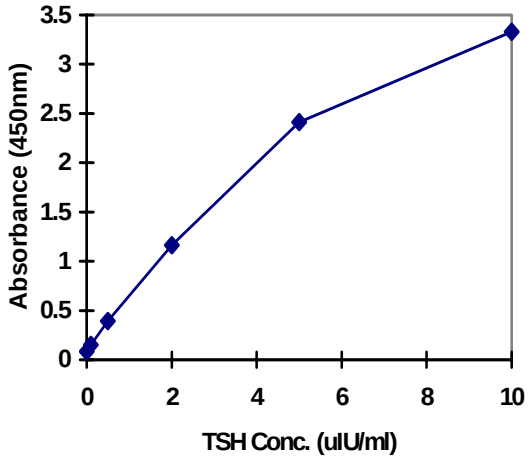
Limitations of the Procedure

1. 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. 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.

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	0.084
0.1	0.152
0.5	0.393
2	1.164
5	2.411
10	3.330



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.

REFERENCES

1. Burger, H. G., Patel, Y. C., Thyrotropin releasing hormone -TSH Clinic. Endocrinol. and Metab., 6, 831-00(1977).
2. Ezrin, C., The Thyroid, S. C. Werner and S. H. lngbar (eds.), Harper and Row, Hagerstown, MD, 9, 174-178 (1978).
3. Pierce, J. G., Endocrinology, 89, 1331-1344 (1971).
4. Berger, S. and Quinn, J. L., Fund. Clin. Chem., N. W. Tietz (ed.), W. B. Saunders Co., Phila., PA 14, 824-848 (1976).
5. Utiger, R. D., The Thyroid, S.C. Werner and S. H. Ingbar (eds.), Harper and Row, Hagerstown, MD, 9, 196-205 (1978).
6. Soos, M. and Siddle, K., J. Immun. Methods, 51, 57-68 (1982).
7. Wada, H. G., Danisch, R. J., Baxter, S. R., et al, Clin. Chem.,28, 1862-1866 (1982).
8. Snyder, P. J. and Utiger, R. D., J. Clin. Endocrinol. Metab., 34, (1972).
9. Engall, E., Methods in Enzymology, Volume 70, Van Vunakis, H. and Langone, J. J. (eds.), Academic Press, New York, 419-492(1980).

Date Adopted	Reference No.
2008-05-01	DA-U-TSH-2008

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ISO 13485-2003

Revision Date: 6/19/08