Annex 1

Instruction for Use

THYROID STIMULATING HORMONE (TSH) ELISA KIT

Catalog No. E1003

INTENDED USE

The Autobio TSH assay is designed for the quantitative determination of thyroid stimulating hormone (TSH) concentration in human serum.

INTRODUCTION

The determination of serum or plasma levels of thyroid stimulation 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 (T4) and triiodothyronine (T3) 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 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.[2] 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 antibodies. The use of a monoclonal antibody in this TSH ELISA test eliminates this interference, 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 TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a specific monoclonal antibody directly against a distinct antigenic determinant on the intact TSH molecule. One monoclonal anti-TSH antibody is used for solid phase immobilization and another 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 incubating 60 minutes at 37℃, the wells are washed with Wash Solution to remove unbound labeled antibodies. Substrate Solution and Chromogen Solution 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, and the color is changed to yellow and the color intensity measured spectrophotometrically at 450 nm. The color intensity is directly proportional to the concentration of TSH in the test sample.

MATERIALS PROVIDED

1. Antibody Coated Microtiter Plate: Microplate with anti-TSH monoclonal antibody (MAB) coated wells (1 plate, 96 wells)
2. Enzyme Conjugate Reagent: Horseradish Peroxidase (HRP) labeled anti-TSH (MAb) in Stabilizing Buffer (1 vial, 6 ml)
3. Reference Standards: 0, 0.5, 2.0, 5.0, 10, 20μIU/ml TSH in Tris solution with preservatives. 1μIU/ml of the reference standard is equivalent to 1μIU/ml of the 2nd IRP 80/558. (6 vials, 1ml/ea)
4. Wash Solution Concentrate: PBS-Tween (1 bottle, 25 ml, 40X)
5. Substrate Solution: Hydrogen Peroxide (1 vial, 7.5 ml)
6. Chromogen Solution: Tetramethylbenzidine (1 vial, 7.5 ml)
7. Stop Solution: 1.0M H_2SO_4 (1 vial, 7.5 ml)

MATERIALS NOT PROVIDED

The following materials are required but not provided in the kit:

1. Distilled water
2. Precision pipettes for delivery of 20-200μl, 100-1000μl (the use of accurate pipettes with disposable plastic tips is recommended)
3. Microplate ELISA reader with a bandwidth of 10nm or less and an optical density range of 0-3.5 OD or greater at 450nm wavelength
4. Magnetic stirrer
5. Vortex Mixer or equivalent
6. Washer for microplates
7. Quality control specimens
8. Incubator
9. Absorbent paper

STORAGE OF TEST KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2-8℃ upon receipt and the microplate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (One year from the date of manufacture). Refer to the package label for the expiration date.
2. Opened test kits will remain stable for at least two months, provided it is stored as prescribed above.

SPECIMEN COLLECTION AND PREPARATION

1. Serum is the recommended sample type for this assay.
2. Collect all blood samples observing universal precautions for venipuncture.
3. Allow samples to clot for 1 hour before centrifugation.
4. Avoid grossly hemolytic, lipemic or turbid samples.
5. Prior to use, specimens should be capped and stored up to 48 hours at 2-8℃. For longer storage, freeze the specimens at -20℃. Thawed samples must be mixed prior to testing.

PRECAUTIONS AND WARNINGS

1. For *in vitro* diagnostic use only.
2. Handling of reagents, serum specimens should be in accordance with local safety procedures.
3. All animal products and derivatives have been collected from healthy animals. Bovine components originate from countries where BSE has not been reported. Nevertheless, components containing animal substances should be treated as potentially infectious.
4. Avoid any skin contact with all reagents, Stop Solution contains H_2SO_4, in case of contact, wash thoroughly with water.
5. Sodium azide in reference standards can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.
6. Do not smoke, drink, eat or apply cosmetics in the working area. Do not pipette by mouth. Use protective clothing and disposable gloves.

REAGENT PREPARTION

1. All reagents should be brought to room temperature (18-25℃) before use.
2. Adjust the incubator to 37℃.
3. Prepare Wash Solution: add 25ml of Wash Solution Concentrate to 1000ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at R.T. for two months.

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that no more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50 µl of standards, specimens, and controls into appropriate wells.
3. Dispense 50 µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this step.
5. Incubate at 37℃ for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with wash solution.
8. Strike the wells sharply onto absorbent paper to remove all residual water droplets.
9. Dispense 50 µl of Chromogen Solution into each well.
10. Dispense 50 µl of Substrate Solution into each well. Gently mix for 15 seconds.
11. Incubate at room temperature in the dark for 20 minutes without shaking.
12. Stop the reaction by adding 50 µl of Stop Solution to each well.
13. Gently mix for 15 seconds. It is important to make sure that blue color changes to yellow completely.
14. Read absorbance at 450nm with a Microplate ELISA reader within 15 minutes.

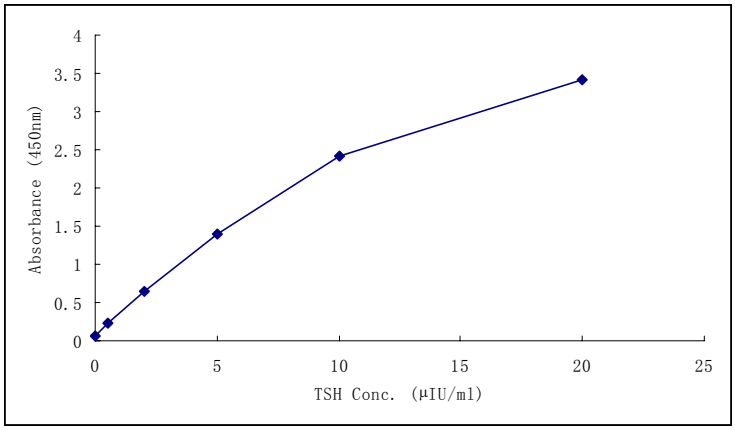
CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On linear graph paper plot the absorbance values (ordinate) for each calibrator against the corresponding concentration of TSH in µIU/ml (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolation on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

EXAMPLE OF STANDARD CURVE

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

TSH (µIU/ml)	Absorbance (450nm)
0	0.062
0.5	0.232
2	0.652
5	1.396
10	2.418
20	3.425



EXPECTED VALUES

Each laboratory should establish its own normal range based on patient population. These values are given only for guidance.

	Normal	Hypothyroid	Hyperthyroid
Number	140	30	80

Mean TSH (μIU/ml)	1.80	11.2	0.12
Range (μIU/ml)	0.4-5.5	>5.2	<0.2

PERFORMANCE

A. Sensitivity

Twenty zero standards were assayed along with a set of other standards. The detection limit, defined as the apparent concentration corresponding to two standard deviations above the average absorbance at zero binding, was not higher than 0.05μIU/ml.

B. Specificity

The cross-reactivity of the TSH assay kit with LH, FSH and HCG was determined by adding these hormones to zero standards. The color intensity produced was then determined.

Hormone Tested	**Concentration**	**Measured Value（μIU/ml）**
hCG	25000 mIU/ml	<0.05
FSH	500 mIU/ml	<0.05
LH	500 mIU/ml	<0.05

C. Precision

a. Intra-Assay Precision

Intra-Assay Precision was determined by assaying 20 replicates of each of 2 sera; low and high.

Serum	**Number**	**Mean**	**SD**	**RSD（%）**
Low	20	0.82	0.054	6.54
High	20	3.63	0.188	5.19

b. Inter-Assay Precision

Inter assay Precision was determined by assaying duplicates of 2 serum pools in 20 separate runs, using a standard curve constructed for each run.

Serum	**Number**	**Mean**	**SD**	**RSD（%）**
Low	20	0.79	0.065	8.23
High	20	3.72	0.274	7.36

D. Accuracy

For samples in the range of 0.2 to 67μIU/ml, the relationship between the Autobio TSH ELISA and the Bayer ACS:180 TSH assay, and the relationship between the Autobio TSH ELISA and ABBOTT ARCHITECT TSH assay are described by the equation:

Method	**No. of Specimens**	**Linear Equation**	**Correlation Coefficient**
Bayer ACS180	200	y = 0.9408x + 0.045	0.979
Abbott ARCHITECT	320	y = 0.9198x - 0.2892	0.973

E. Hook effect

A sample spiked with TSH up to 4000μIU/ml gives higher absorbance than the last standard point.

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.

Heterophilic antibodies in human serum can react with reagent immunoglobulin, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.

Serum TSH concentration is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function and the responsiveness of pituitary to TRH. Thus, thyrotropin concentration alone is not sufficient to assess clinical status.

For diagnostic purposes, the results obtained form this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

SYMBOLS

	**BATCH CODE**
	**USE BY**
	**MANUFACTURER**
	**CONTAINS SUFFICIENT FOR <n> TESTS**
	***IN VITRO* DIAGNOSTIC MEDICAL DEVICE**
	**TEMPERATURE LIMITATION**
	**CATALOGUE NUMBER**
	**CONSULT INSTRUCTIONS FOR USE**

REFERENCES:

[1]Walker W.H.C. Introduction: An Approach to Immunoassay. Clin. Chem. 1977; 23: 384.
[2]Kirkegaard C., Friis T. and Siersback-Nielsen K. Acta Endocrinol. 1974; 77: 71.
[3]Wisdom G.B. Enzyme-Immunoassay. Clin. Chem. 1976; 22: 1243.
[4]Hoffenberg R. Medicine 1978; 8: 392.
[5]Lieblich J., Utiger R.D. J. Clin. Invest. 1972; 51: 1939.

For order and inquires, please contact

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