
THYROID STIMULATING HORMONE (TSH) CHEMILUMINESCENCE

IMMUNOASSAY KIT

Catalog No. CL1003-2

INTENDED USE

The Autobio Thyroid Stimulating Hormone (TSH) Chemiluminescence Immunoassay (CLIA) Kit 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 CLIA 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 CLIA 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 for 60 minutes at 37°C, the wells are washed with Wash Solution to remove unbound labeled antibodies. Upon the addition of the substrate, the horseradish peroxidase activity bound on the wells is then assayed by a chemiluminescence reaction. The Related Light Unit (RLU) 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, 48wells/96 wells)
2. Enzyme Conjugate Reagent: Horseradish Peroxidase (HRP) labeled anti-TSH (MAb) in Stabilizing Buffer (1 vial, 3.0 ml/6.0ml)
3. Reference Standards: 0.25, 0.5, 2.0, 10, 30, 60 μ IU/ml TSH in Tris solution with preservatives. 1 μ IU/ml of the reference standard is equivalent to 1 μ IU/ml of the 2nd IRP 80/558. (6 vials, 1.0ml/ea)
4. PBS-T powder: PBS-Tween (1bag, 5g)
5. Substrate A: (1 vial, 3.5ml/6.0ml)
6. Substrate B: (1 vial, 3.5ml/6.0ml)

MATERIALS NOT PROVIDED

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

1. Distilled water

2. Precision pipettes for delivery of 20-200 μ l, 100-1000 μ l (the use of accurate pipettes with disposable plastic tips is recommended)
3. Luminometer
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 $^{\circ}$ C upon receipt and the microtiter plate 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 $^{\circ}$ C. For longer storage, freeze the specimens at -20 $^{\circ}$ C. 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.
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 PREPARATION

1. All reagents should be brought to room temperature (18-25 $^{\circ}$ C) prior to use.
2. Adjust the incubator to 37 $^{\circ}$ C
3. Prepare Wash Solution: add 1 bag of PBS-T powder to 500ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at room temperature for 2 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 RLU readings.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50 μ l of Enzyme Conjugate Reagent into each well.
3. Dispense 100 μ l of standards, specimens, and controls into appropriate well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing in this step.

5. Incubate at 37°C for 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter plate 5 times with wash solution.
8. Strike the wells sharply onto absorbent paper to remove all residual water droplets.
9. Dispense 50µl of Substrate A, then 50µl of Substrate B into each well. Gently mix for 10 seconds.
10. Put the microplate into the detecting chamber of Luminometer for 5 minutes, then read the RLU values of each well.

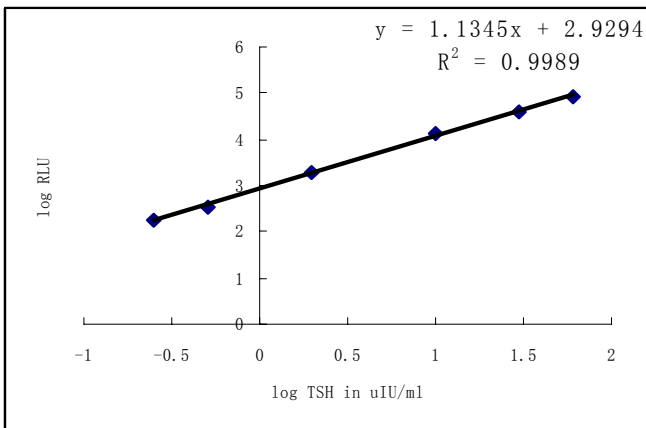
CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On logarithmic graph paper, Plot the log₁₀RLU of each reference standard against the common logarithm of corresponding TSH concentration in µIU/ml with log₁₀RLU on the Y-axis and log₁₀Conc. on the X-axis. Connect the reference standard points with a curved line.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a linear regression 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)	RLU
0.25	179.2
0.5	356.8
2	1898.9
10	13227.2
30	40745.7
60	80417.9



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	157	40	70
Mean TSH (µIU/ml)	1.49	13.6	0.19
Range (µIU/ml)	0.35-5.3	>5.5	<0.3

PERFORMANCE

A. Sensitivity

Twenty zero standards were assayed along with a set of other standards. The sensitivity, defined as the apparent concentration corresponding to two standard deviations above the average RLU at zero binding, was lower than 0.08µIU/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 RLU was then determined.

Hormone Tested	Concentration	Measured Value ($\mu\text{IU/ml}$)
hCG	2500mIU/ml	<0.05
FSH	500mIU/ml	<0.05
LH	500mIU/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	0.79	0.052	6.64
High	20	3.92	0.17	4.31

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	0.97	0.072	7.42
High	20	3.89	0.23	5.92

D. Accuracy

For samples in the range of 0.2 to 67 $\mu\text{IU/ml}$, the relationship between the Autobio TSH CLIA and the Bayer ACS:180 TSH assay, and the relationship between the Autobio TSH CLIA and ABBOTT ARCHITECT TSH assay are described by the equation:

Method	No. of Specimens	Linear Equation	Correlation Coefficient
Bayer ACS180	180	$y = 0.9514x + 0.089$	0.958
Abbott ARCHITECT	320	$y = 0.9346x - 0.317$	0.977

E. Hook effect

A sample spiked with TSH up to 4000 $\mu\text{IU/ml}$ gives higher RLU 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 from 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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