

THYROID STIMULATING HORMONE (TSH) ENZYME IMMUNOASSAY TEST KIT

Catalog Number: 6107225



ENZYME IMMUNOASSAY FOR THE QUANTITATIVE DETERMINATION OF THYROID STIMULATING HORMONE (TSH) IN HUMAN SERUM

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

TSH Enzyme Immunoassay

INTENDED USE

For the quantitative determination of thyroid stimulating hormone (TSH) concentration in human serum. The assay is useful in the diagnosis of thyroid or pituitary disorders.

INTRODUCTION

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 daltons and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism (1,2). Increase in serum concentrations of TSH, which is primarily responsible for the synthesis and release of thyroid hormones, is an early and sensitive indicator of decrease thyroid reserve and in conjunction with decreased thyroxine (T4) concentrations is diagnostic of primary hypothyroidism. The expected increase in TSH concentrations demonstrates the classical negative feedback system between the pituitary and thyroid glands. That is, primary thyroid gland failure reduces secretion of the thyroid hormones, which in turn stimulates the release of TSH from the pituitary.

Additionally, TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduces the response of the pituitary to the stimulatory effects of TRH. In secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH levels are generally low or normal. Either pituitary TSH deficiency (secondary hypothyroidism) or insufficiency of stimulation of the pituitary by TRH (tertiary hypothyroidism) causes this. The TRH stimulation test differentiates these conditions. In secondary hypothyroidism, TSH response to TRH is blunted while a normal or delayed response is obtained in tertiary hypothyroidism.

Further, the advent of immunoassays has provided the laboratory with sufficient sensitivity to enable the differentiating of hyperthyroidism from euthyroid population and extending the usefulness of TSH measurements. This method

is a second-generation assay, which provide the means for discrimination in the hyperthyroid-euthyroid range. The functional sensitivity (<20% between assay CV) of the one hour procedure is 0.195 mIU/L while the two hour procedure has a functional sensitivity of 0.095 mIU/L (3).

In this method, TSH calibrator, patient specimen or control is first added to a anti TSH coated well. Monoclonal and enzyme labeled antibodies are added and the reactants mixed. Reaction between the various TSH antibodies and native TSH forms a sandwich complex that binds with the anti-TSH coated well.

After the completion of the required incubation period, the antibody bound enzyme-thyrotropin conjugate is separated from the unbound enzyme-thyrotropin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known thyrotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with thyrotropin concentration.

PRINCIPLE OF THE TEST

Immunoassay:

The essential reagents required for an immunoassay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, in excess, and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of anti-TSH coated on the well and exogenously added monoclonal anti-TSH antibody.

Upon mixing a reaction takes place between fixed monoclonal antibody, the enzyme-labeled second antibody and a serum containing the native antigen. The reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a sandwich complex.

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration.

By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

REAGENTS

Materials provided with the kit:

- **Microwell plate.** 12x8 well strips. Individually separable wells. Murine Monoclonal Anti-TSH-coated, packaged in an aluminum bag with a drying agent.
- **Calibrators.** 6 Vials x 0.4 mL. Concentrations: 0(A), 0.25(B), 0.75(C), 2(D), 5(E) and 15(F) mIU/L.
- **Control.** 1 x 0.4 mL. The concentrations are started on the label.



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- **Enzyme Conjugate.** TSH-horseradish peroxidase (HRP) conjugate. 12 mL.
- **Wash Solution Concentrate.** Surfactant in phosphate buffered saline. 25 mL.
- **TMB-Substrate.** H₂O₂-TMB 0.25 g/L (avoid any skin contact). 12 mL
- **Stop Solution.** Sulphuric acid 0.15 mol/L (corrosive: avoid any skin contact). 12 mL

PRECAUTIONS

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells. Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) can not be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 mL of the specimen is required.

Materials required but not provided:

- Multichannel pipettes and micropipettes (Precision $\geq 1.5\%$) and disposable tips.
- Microplate reader with a 450 nm filter. Reference filter of 620 or 655 nm is advisable.
- Manual or automated wash system.
- Absorbent paper of blotting the microplate wells.
- Distilled or deionised water.
- Timer.

REAGENT PREPARATION

- **Wash Buffer.** Dilute contents of Wash Concentrate to 1000 mL with distilled or deionized water in a suitable storage container. Store at room temperature.

ASSAY PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 30°C).

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
 2. Pipette **0.025 mL (25 µL)** of the appropriate serum reference, control or specimen into the assigned well.
 3. **Incubate 10 minutes at room temperature !**
 4. Add **0.100 mL (100 µL)** of the conjugate to each well.
 5. Swirl the microplate gently for 10 seconds to mix and cover.
 6. Incubate **90 minutes** at room temperature.
 7. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
 8. Add **app. 300µL** of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
 9. Add **0.100 mL (100 µL)** of TMB/substrate solution to all wells. Always add reagents in the same order to minimize reaction time differences between wells.
 10. Incubate at room temperature for **20 minutes**.
 11. Add **0.100 mL (100 µL)** of stop solution to each well .
- Always add reagents in the same order to minimize reaction time differences between wells.**
12. Read the absorbance in each well at 450 nm in a microplate reader.

The results should be read within five minutes after adding the stop solution. Preferably readings should take place immediately after stopping the reaction since the OD450 may slightly decrease with the course of time.

QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. Other parameters that should be monitored include the 80, 50 and 20% intercepts of the dose response curve for run-to-run reproducibility. In addition, maximum absorbance should be



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consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of thyrotropin in unknown specimens.

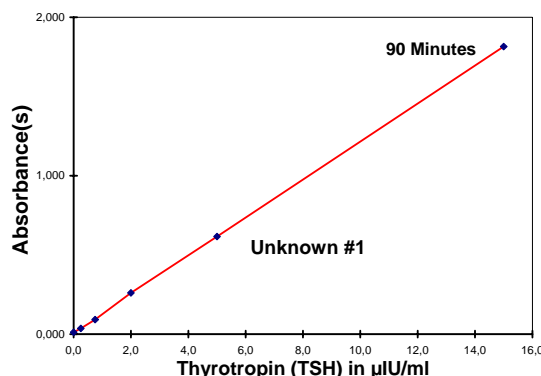
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding TSH concentration in mIU/L on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of TSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/L) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.019) intersects the dose response curve at (15.3 mIU/L) TSH concentration (See Figure 1).

Note: Computer data reduction software designed for IEMA assays may also be used for the data reduction.

EXAMPLE 1

INCUBATION TIME		90 MINUTE		
WELL	SERUM REFERENCES	ABSORBANCE		
1	0.0 mIU/L	0.014		
2	0.0 mIU/L	0.011		
3	0.25 mIU/L	0.031		
4	0.25 mIU/L	0.037		
5	0.75 mIU/L	0.091		
6	0.75 mIU/L	0.077		
9	2 mIU/L	0.271		
10	2 mIU/L	0.259		
13	5 mIU/L	0.598		
14	5 mIU/L	0.633		
15	15 mIU/L	1.853		
16	15 mIU/L	1.776		
	Unknown	Avg.		
WELL	I. D.	O.D.	O.D.	Value
15	Unknown #1	0.412		
16	Unknown #1	0.424	0.418	3.6 mIU/L

Figure (1)



*The data presented in Example 1 and Figure 1 are for illustration only and should not be used in lieu of a dose response curve prepared with each assay.

Q.C. PARAMETERS

Maximum Absorbance (15mIU/L) = 1.4 - 2.8

LIMITATIONS OF THE PROCEDURE

1. Assay Performance.

It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift. If more than one (1) plate is used, it is recommended to repeat the dose response curve.

Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipemic or hemolysed specimen(s) should similarly not be used.

Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells.

Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.

Patient specimens with TSH concentrations above 40 mIU/L may be diluted with the zero calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor. Each component in one assay should be of the same lot number and stored under identical conditions.

2. Interpretation.

If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted



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values for the calibrators fall within 10% of the assigned concentrations.

Serum thyrotropin 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.

Serum thyrotropin values may be elevated by pharmacological intervention. Domperidone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.

A decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxine (4).

Genetic variations or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results due to the reactivity of the antibodies involved

3. "NOT INTENDED FOR NEWBORN SCREENING"

EXPECTED VALUES

A study of euthyroid adult population was undertaken to determine expected values for the TSH IEMA Microplate Test System. The number and determined range are given in Table 1. A nonparametric method (95% Percentile Estimate) was used.

TABLE 1
(in mIU/L)

Number	139
Low Normal Range	0.39
High Normal Range	6.16
70% Confidence Intervals for 2.5 Percentile	
Low Range	0.29 – 0.54
High Range	5.70 – 6.92

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 upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon 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.

PERFORMANCE CHARACTERISTICS

A. Precision

The within and between assay precision of the TSH IEMA Microplate Test System were determined by analyses on two different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2

Within Assay Precision (Values in mIU/L)

Sample	N	X	S.D.	C.V.
Pool 1	24	2.32	0.07	2.98%
Pool 2	24	5.00	0.11	2.27%
Pool 3	24	14.55	0.34	2.34%

TABLE 3

Between Assay Precision* (Values in mIU/L)

Sample	N	X	S.D.	C.V.
Pool 1	10	2.13	0.11	8.1%
Pool 2	10	13.93	0.45	4.1%

*As measured in ten experiments in duplicate over seven days.

B. Accuracy

This TSH Microplate IEMA Test System was compared with a reference immunochemiluminescence assay. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.01 mIU/L – 41 mIU/L). The total number of such specimens was 165. The least square regression equation and the correlation coefficient were computed for the TSH IEMA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Least Square

Method	Mean (x)	Regression Analysis	Correlation Coefficient
This Method	4.55	$y = 0.47 + 0.968(x)$	0.995
Reference	4.22		

Only slight amounts of bias between the IEMA TSH system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

C. Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0 mIU/L serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose:

For 90 min incubation = 0.027 mIU/L.

D. Specificity

The cross-reactivity of the thyrotropin IEMA method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations, The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyrotropin needed to produce the same absorbance.



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Substance	CrossReactivity	Concentration
Thyrotropin (hTSH)	1.0000	-
Follicle Stimulating Hormone (hFSH)	< 0.0001	1000 ng/mL
Luteinizing Hormone (hLH)	< 0.0001	1000 ng/mL
Chorionic Gonadotropin (hCG)	< 0.0001	1000 ng/mL

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