![MP Biomedicals logo]

MP Biomedicals
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TSH ChLIA Kit
Catalog No.: 07M375A, 07M375B

INTENDED USE

The MP Biomedicals TSH (Thyroid Stimulating Hormone; Thyrotropin) ChLIA Kit is intended to be used for the quantitative determination of TSH Concentration in Human Serum by a Microplate Enzyme Immunoassay, Chemiluminescence (ChLIA). This test is for in vitro diagnostic use only.

SUMMARY AND EXPLANATION OF THE TEST

Measurement of the serum concentration of TSH (Thyroid Stimulating Hormone; Thyrotropin), 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] The structure of human TSH is similar to that of the pituitary and placental gonadotropins, consisting of an 89-amino acid α-subunit which is similar or identical between these hormones and a 115-amino acid β-subunit, which apparently confers hormonal specificity. The production of the 2 subunits is separately regulated with apparent excess production of the α-subunit. The TSH molecule has a linear structure consisting of the protein core with carbohydrate side chains; the latter accounts for 16% of the molecular weight.

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 reduce 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 immunoenzymetric assays 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 provides the means for discrimination in the hyperthyroid-euthyroid range.

In this method, TSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (Abs) are added and the reactants mixed. Reaction between the various TSH antibodies and native TSH forms a sandwich complex that binds with the streptavidin coated to the 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 light.
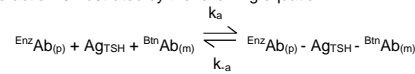
The employment of several serum reference calibrators of known thyrotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with thyrotropin concentration.

PRINCIPLE

Immunoenzymometric assay (Type 3)

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme conjugated 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 streptavidin coated on the well and exogenously added biotinylated monoclonal anti-TSH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or stearic hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + Ag_{TSH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Ab (Excess Quantity)
Ag_{TSH} = Native Ag (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Polyclonal Ab (Excess Quantity)
$^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through te high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)} - Ag_{TSH} - {}^{Btn}Ab_{(m)}$ + Streptavidin$_{C.W.}$ ⟹ immobilized complex
Streptavidin$_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface
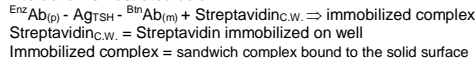
After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity, determined by reaction with a substrate that generates light, in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum reference calibrators 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:
A. TSH Calibrators – 1 mL/vial
Seven (7) vials of references for TSH Antigen at levels of 0 (**A**), 0.5 (**B**), 2.5 (**C**), 5.0 (**D**), 10 (**E**), 20 (**F**) and 40 (**G**) μIU/mL. A preservative has been added. Store at 2-8°C.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IRP 80/558.
B. TSH Tracer Reagent – 13 mL/vial
One (1) vial containing enzyme labeled affinity purified polyclonal goat antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.
C. Light Reaction Wells – 96 wells
One 96-well white microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
D. Wash Solution Concentrate – 20 mL/vial
One (1) vial containing a surfactant in Buffered Saline. A preservative has been added. Store at 2-8°C.
E. Signal Reagent A – 7 mL/vial
One (1) vial containing luminol in buffer. Store at 2-8°C.
F. Signal Reagent B – 7 mL/vial
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.
G. Package Insert.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

Required But Not Provided:
1. Pipette(s) capable of delivering 0.050 and 100 mL (50 & 100 μL) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350 mL (100 & 350 μL) volumes with a precision of better than 1.5% (optional).
3. Microplate washer or a squeeze bottle (optional).
4. Microplate luminometer.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Storage container for storage of wash buffer.
10. Distilled or deionized water.
11. Quality Control Materials.

PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

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.

Safe Disposal of kit components must be according to local regulatory and statutory requirements.

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 with or without 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 use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 mL (100 μL) of the specimen is required.

REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of Wash Concentrate to 1000 mL with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.
2. **Working Signal Reagent Solution -** Store at 2 - 8°C.
Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 mL of A and 1 mL of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, reference calibrators and controls to room temperature (20 - 27° C).
Test Procedure should be performed by a skilled individual or trained professional

1. Format the microplates' wells for each serum reference calibrator, 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 0.050 mL (50 μL) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 mL (100 μL) of the TSH Tracer Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 45 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
7. Add (0.350 mL) 350 μL 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 four (4) additional times.**
8. Add 0.100 mL (100 μL) of working signal reagent solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate for five (5) minutes at room temperature in the dark.
10. Read the RLUs (*Relative Light Units*) in each well in a microplate luminometer for at least 0.2seconds per well. **The results can be read within 30 minutes of adding the signal solution.**

QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated ranges for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control records should be maintained and used to monitor batch to batch consistency.

CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of TSH in unknown specimens.
1. Record the RLUs obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLUs for each duplicate serum reference calibrator versus the corresponding TSH concentration in μIU/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of TSH for an unknown, locate the average RLUs for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in μIU/mL) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLUs (25677) of the unknown intersects the calibration curve at 7.1 μIU/mL TSH concentration (See Figure 1).

Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	RLU (A)	Mean RLU (B)	Value (µIU/mL)
Cal A	A1	100	102	0
	B1	105		
Cal B	C1	1290	1325	0.5
	D1	1350		
Cal C	E1	7663	7631	2.5
	F1	7600		
Cal D	G1	17878	17761	5.0
	H1	17645		
Cal E	A2	36315	35231	10.0
	B2	34147		
Cal F	C2	61811	62071	20.0
	D2	62331		
Cal G	E2	99820	100000	40.0
	F2	100180		
Ctrl 1	G2	907	905	0.34
	H2	902		
Ctrl 2	A3	21870	21669	6.0
	B3	21468		
Sample	C3	26231	25677	7.1
	D3	25124		

The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLU's of the calibrators have been normalized to 100,000 RLU's for the G calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light.

Figure 1
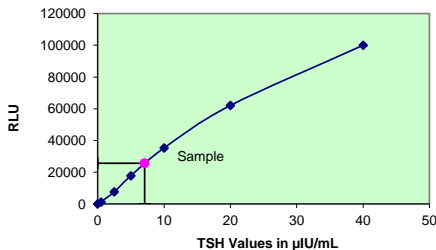
LIMITATIONS OF PROCEDURE

Assay Performance
1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from MP Biomedicals' IFU may yield inaccurate results.
9. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.

10. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

Interpretation
1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, MP Biomedicals shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Serum TSH concentration is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. **Thus, TSH concentration alone is not sufficient to assess clinical status.**
8. Serum TSH values may be elevated by pharmacological intervention. Domperidone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.
9. A decrease in TSH values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxine.[4]
10. Genetic variations or degradation of intact TSH into subunits may affect the biding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due to the reactivity of the antibodies involved.

"NOT INTENDED FOR NEWBORN SCREENING"

EXPECTED RANGES OF VALUES

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

TABLE I
Expected Values for the TSH ChLIA (in µIU/mL)

Number	85
Low Normal Range	0.42
High Normal Range	5.45
70% Confidence Intervals for 2.5 Percentile	
Low Range	0.30 – 0.55
High Range	5.05 – 6.02

It is important to keep in mind that expected values for normal population 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

Precision
The within and between assay precision of the TSH ChLIA test were determined by analyses on three 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 µIU/mL)

Sample	N	X	σ	C.V.%
Level 1	20	0.26	0.03	11.9
Level 2	20	5.15	0.27	5.3
Level 3	20	32.00	2.15	6.7

TABLE 3
Between Assay Precision* (Values in µIU/mL)

Sample	N	X	σ	C.V.%
Level 1	20	0.35	0.05	13.9
Level 2	20	5.42	0.52	9.6
Level 3	20	37.18	2.14	5.8

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

Sensitivity
The sensitivity (detection limit) was ascertained by determining the variability of the 0 µIU/mL serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose. It was determined to be 0.0062 µIU/mL.

Accuracy
The TSH ChLIA test was compared with a reference assay. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.01 µIU/mL – 41 µIU/mL). The total number of such specimens was 181. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	14.97	y = 1.15 + 0.956 (x)	0.973
Reference	14.44		

Only slight amounts of bias between TSH ChLIA test 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.

Specificity
The cross-reactivity of this 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 light intensity.

Substance	Cross Reactivity	Concentration
Thyrotropin (hTSH)	1.0000	-
Follitropin (hFSH)	< 0.0001	1000 ng/mL
Lutropin Hormone (hLH)	< 0.0001	1000 ng/mL
Chorionic Gonadotropin (hCG)	< 0.0001	1000 ng/mL

REFERENCES

1. Hopton M.R., & Harrap, J.J., "Immunoradiometric assay of thyrotropin as a "first line thyroid function test in the routine laboratory", Clinical Chemistry" 32, 691. (1986)
2. Caldwell, G. et. Al., "A new strategy for thyroid function testing", Lancet I, 1117. (1985)
3. Young, D.S., Pestaner, L.C., and Gilberman, U., "Effects of Drugs on Clinical Laboratory Tests." Clinical Chemistry 21, 3660. (1975)
4. Spencer, CA, et al., "Interlaboratory/Intermethod differences in Functional Sensitivity of Immunometric Assays of Thyrotropin (TSH) and Impact on Reliability of Measurement of Subnormal Concentrations of TSH", Clinical Chemistry 41, 367. (1995)
5. Braverman, LE.: "Evaluation of thyroid status in patients with thyrotoxicosis." Clin. Chem. 42, 174-178. (1996)
6. Braverman, LE., Utigen, RD., Eds.: Werner and Ingbar's 'The Thyroid – A Fundamental and Clinical Text' 7th Ed. Philadelphia. Lippinscott-Raven. (1996)
7. Degroot, LJ, Larsen, PR., Hennemenn, G.: Eds.'The Thyroid and its Diseases.' 6th Ed. New York. Churchill Livingstone. (1996)
8. Fisher, DA.:"Physiological variations in thyroid hormones: Physiological and Pathophysiological considerations." Clin. Chem. 42, 135-139. (1996)
9. Beck-Peccoz, P., Persani, L.: "Variable biological activity of thyroid stimulating hormone." Eur.J.Endo 131,331-340. (1994)
10. Becker, DV., Bigos, ST., Gaitan, E.: 'Optimal use of blood tests for assessment of thyroid function." JAMA 269, 2736-2740. (1989)
11. Fisher DA, Klein AH: 'Thyroid development and disorders of thyroid function in the newborn.' NEJM. 304, 702-712. (1981)
12. Burtis CA. Ashwood ER (Ed): Tietz Textbook of Clinical Chemistry. 2nd. Ed. WB Saunders Company. Philadelphia, p 2208. (1994)
13. Alseveir RN, Gotlin RW: Handbook of Endocrine Tests in Adults and Children. 2nd. Ed. Yearbook Publication. Chicago pg. 22-25. (1978)
14. Magner JA: Thyroid Stimulating Hormone; Biosynthesis, Cell Biology and bioactivity. Endo. Review 11, 35. 385. (1990)