



## Human Thyroid Stimulating Hormone (TSH) MICRO-ELISA Test Kit

**Prod. No.:** T112  
**Pkg. Size:** 96 Tests

### Description

The MICRO-ELISA THYROID STIMULATING HORMONE (TSH) test is a solid phase sandwich-type enzyme immunoassay (ELISA) for the in vitro quantitative determination of TSH concentration in human serum.

### Summary and Explanation of the Test

The principal tests used in the laboratory evaluation of thyroid function are Total Thyroxine ( $T_4$ ), Total Triiodothyronine ( $T_3$ ), T-Uptake (T-Up), a calculated Free Thyroxine Index (FTI) and Thyroid Stimulating Hormone (TSH). The results of these tests are interrelated and help the clinician in making a diagnosis. Clinical hypothyroidism results from underproduction of thyroid hormones by the thyroid gland, consequently an abnormally low circulating  $T_4$  and  $T_3$  concentration in blood. Clinical hyperthyroidism results from excessive production of thyroid hormones and resulting elevation of  $T_4$  and  $T_3$  concentrations.

The manifestations of thyroid dysfunction can result from disease of the thyroid gland (PRIMARY hyperthyroidism or hypothyroidism), disease of the pituitary gland (SECONDARY hyperthyroidism or hypothyroidism) or disease of the hypothalamus (TERTIARY hyperthyroidism or hypothyroidism).

Thyroxine (3,5,3',5'-tetraiodo-L-thyronine,  $T_4$ ) and Triiodothyronine (3,5,3'-triiodo-L-thyronine,  $T_3$ ), are the hormones originating from the thyroid gland.  $T_4$  and  $T_3$  are responsible for regulating diverse biochemical processes throughout the body that are essential for protein synthesis, normal development, metabolic and neural activity.

$T_4$  is synthesized within the thyroid gland and secreted directly into the bloodstream. Approximately 30% of the circulating  $T_4$  is enzymatically deiodinated at the 5' position in the peripheral tissues to yield  $T_3$ . The  $T_4$  likely serves as a "prohormone" for  $T_3$ , which has a much greater metabolic activity.

$T_4$  and  $T_3$  are transported through the peripheral blood stream largely bound to serum proteins. The major transport protein is Thyroxine Binding Globulin (TBG) which normally accounts for 80% of the bound hormone. The other thyroid hormone binding proteins are Thyroxine Binding Prealbumin and Albumin. Only about 0.3% of the total serum  $T_3$  and only about 0.1% of the total serum  $T_4$  are unbound and free to diffuse into tissue to exert their biological effects. When the level of TBG increases, the level of total  $T_4$  will increase to maintain the same level of unbound or free  $T_4$  in the bloodstream of an euthyroid individual.

Simply determining the total  $T_4$  concentration fails to take into account the variations in TBG levels that affect the unbound thyroxine (free  $T_4$ ) concentration. TBG levels can vary for reasons incidental to the patient's thyroid status such as the presence of certain drugs, steroid hormones, pregnancy, and

various non-thyroidal diseases. The Thyroid Uptake (T-Up) test is an indirect measurement of empty binding sites for  $T_4$  on the TBG molecule (unsaturated TBG) in the patient specimen. The number obtained from the multiplication of the Total  $T_4$  concentration by the Thyroid Uptake value is called a Free Thyroxine Index (FTI). The FTI correlates more closely with Free  $T_4$  (the metabolic active fraction) concentration than does the total  $T_4$  concentration alone. The FTI is therefore a better method of monitoring thyroid function and diagnosing thyroid illness than is a Total  $T_4$  determination alone.

Diseases of the thyroid gland can result in clinical signs of thyroid dysfunction. Primary hypothyroidism results in underproduction of  $T_4$  by the thyroid gland and consequently an abnormally low circulating  $T_4$  concentration in the blood. Primary hyperthyroidism leads to excessive thyroid production of  $T_4$  and a resulting elevated  $T_4$  concentration.

The determination of total serum  $T_3$  is used in the differential diagnosis of thyroid disease, particularly hyperthyroidism. In most hyperthyroid patients, both serum  $T_3$  and  $T_4$  are elevated. However, approximately 5-10% of hyperthyroid patients have elevated  $T_3$  concentrations but normal serum  $T_4$ , a condition known as  $T_3$ -thyrotoxicosis. Such clinical conditions make it vital to establish that serum  $T_3$  is normal before excluding the diagnosis of hyperthyroidism. Serum  $T_3$  level is also an excellent indicator for the ability of the thyroid to respond to both stimulatory and suppressive tests.

The thyroid gland function is regulated by the level of Thyroid Stimulating Hormone (TSH) which is produced and secreted by the pituitary gland. TSH is produced by the anterior lobe of the pituitary gland and acts on the thyroid gland to release thyroid hormones. The release of TSH from the pituitary is regulated by the hypothalamus when it secretes TRH (thyrotropin releasing hormone).

In an euthyroid individual, the levels of thyroid hormones in the blood are inversely related to the levels of TSH and TRH. When the levels of thyroid hormones rise, the levels of TRH and TSH fall; and when the levels of thyroid hormones fall, the levels of TRH and TSH rise. In the event of failure of the thyroid gland, the levels of thyroid hormones fall and the negative feedback results in an elevated level of TSH in the blood. Elevated levels of TSH are thus useful in the diagnosis of primary hypothyroidism. Conversely, in the case of primary hyperthyroidism, the elevated levels of thyroid hormones will result in decreased levels of TSH.

When there is a failure of the pituitary or the hypothalamus (secondary or tertiary hypothyroidism), the level of TSH is decreased in the presence of low levels of thyroid hormones. In secondary or tertiary hyperthyroidism, the level of TSH is increased in the presence of high levels of thyroid hormones.

$T_4$   $T_3$  T-UP FTI TSH



Euthyroid	N	N	N	N	N
Pregnant Euthyroid	I	I	D	N	N
1° Hyperthyroidism	I	I	I	I	D
2° or 3° Hyperthyroidism	I	I	I	I	I
T <sub>3</sub> Thyrotoxicosis	N	I	N	N or I	N or D
1° Hypothyroidism	D	D	D	D	I
2° or 3° Hypothyroidism	D	D	D	D	D

N = Normal; D = Decreased; I = Increased

## Principle of the Procedure

The measurement of TSH is complicated by the chemical structure of the molecule; it is composed of two subunits designated alpha and beta. The alpha subunit, which is similar to the other polypeptide hormones LH, FSH and hCG can cause cross reactivity with some antibodies used in TSH testing. It is the beta subunit of TSH that gives it its unique physiological properties. The MICRO-ELISA TSH is designed to eliminate this cross reactivity by use of two monoclonal antibodies that are specific for unique determinates on the TSH molecule.

The MICRO-ELISA TSH test is based on the principle of a solid phase enzyme-linked immunosorbent assay (ELISA). The assay system utilizes two antibodies (mouse monoclonal) directed against distinct antigenic determinants on the TSH molecule.

Specifically, plastic wells are coated with anti-TSH (mouse monoclonal). With the addition of a test sample or appropriate controls containing TSH, immune complexes are formed between TSH in the sample and the solid phase anti-TSH. Anti-TSH (mouse monoclonal) enzyme-labeled with horseradish peroxidase is added to each well. During an incubation period (45 minutes at room temperature), the TSH molecule is sandwiched between the solid phase and enzyme-labeled antibodies. The sample is then decanted and the wells are washed to remove unbound labeled antibody. An enzyme substrate-chromogen (hydrogen peroxide, H<sub>2</sub>O<sub>2</sub>, and tetramethylbenzidine, TMB) is added to the well and incubated for 30 minutes at room temperature, resulting in the development of a blue color. The addition of 1.0 N H<sub>2</sub>SO<sub>4</sub> stops the reaction and converts the color to yellow and increases the absorbance by a factor of approximately 3. The intensity of the yellow color is directly proportional to the concentration of TSH in the sample. The concentration of TSH in the patient sample is interpolated from a standard curve relating the absorbance, measured spectrophotometrically at 450 nm, of each calibrator to the concentration of TSH.

## Reagents

### Components in Each 96 Test

#### Micro-Elisa TSH Diagnostic Kit

- 96 wells, TSH **ANTIBODY COATED WELLS**: Coated with anti-TSH antibody (mouse monoclonal); contained in a pack with silica gel desiccant.
- 1 bottle, 22 ml, TSH **ENZYME ANTIBODY CONJUGATE**: anti-TSH (mouse monoclonal) labeled with horseradish peroxidase in buffered protein solution; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives; contains FD&C red # 40 as coloring agent.

- 1 bottle, 12 ml, **SUBSTRATE-CHROMOGEN** Buffered hydrogen peroxide and 3,3',5,5'- tetramethylbenzidine (TMB) solution.
- 1 bottle, 12 ml, **STOP SOLUTION** 1 N H<sub>2</sub>SO<sub>4</sub>.
- 1 vial, 4 ml, **0 μIU/ml TSH CALIBRATOR**: Bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives.
- 5 vials, 1 ml, TSH **CALIBRATORS**: TSH in bovine serum; contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. 0.25, 1.0, 5.0, 10 and 20 μIU/ml.
- 1 bottle, 60 ml, **WASH BUFFER CONCENTRATE (20X)**: Buffered detergent solution, contains 0.02% thimerosal and 0.002% gentamicin sulfate as preservatives. Dilute bottle to 1200 ml with deionized water.

## Additional Materials Required

Disposable tip precision pipets - 0.05, 0.1 and 0.2 ml.  
Microtiter plate reader.  
Distilled or deionized water.

## Storage and Stability

Store unopened kits at 2°-8°C. The following components may be stored at ambient temperature: WELLS, SUBSTRATE-CHROMOGEN, WASH BUFFER and STOP SOLUTION. Expiration date printed on the kit indicates limits of stability.

The TSH ANTIBODY COATED WELLS are supplied in a resealable bag containing a desiccant and must be stored with the bag sealed to protect from moisture. Wells can be stored at 2°-30°C.

## Chemical or Physical

### Indications of Instability

Alterations in the physical appearance of reagents, or results consistently outside the acceptable limits for control sera, may be due to reagent contamination or deterioration.

## Instruments

Performance of the MICRO-ELISA TSH test requires use of a precision microtiter plate reader a wavelength of 450 ± 20 nm:

## Specimen Collection and Preparation

Serum samples are used in the MICRO-ELISA TSH Diagnostic Kit procedure. No special preparation of the patient is necessary; fasting is not required. Repeated freezing and thawing of specimens should be avoided. No additives or preservatives are necessary.

**STORAGE:** Specimens may be stored in a tightly sealed tube at 2°-8°C for two days. If the serum is not assayed within 2 days, store frozen (-20°C) in a tightly sealed tube for up to 3 weeks. Specimens should be allowed to come to room temperature and should be mixed thoroughly by gentle inversion before assaying.



Do not use grossly lipemic specimens. Moderately lipemic, hemolyzed and icteric specimens should not interfere with the assay.

## Micro-Elisa TSH Procedure

### Reagent Preparation

Dilute bottle of WASH BUFFER CONCENTRATE (20X) solution to 1200 ml with deionized water. Diluted wash solution will be stable until the expiration date stamped on the kit.

### Preliminary Comments And Precautions

Patient sample may contain pathogens: treat all samples as potentially infectious.

Reagents contain thimerosal; avoid contact with skin.

Avoid contact with SUBSTRATE-CHROMOGEN (tetramethyl-benzidine) solution. It is harmful if inhaled or absorbed through skin (may cause irritation).

CAUTION: Source material used to prepare Calibrators was derived from human material. The material was tested using FDA-approved methods and found non-reactive for Hepatitis B Surface Antigen (HBsAg) by ELISA and non-reactive for HIV by ELISA. No known test method can offer total assurance that infectious agents are absent. HANDLE THESE REAGENTS AS IF THEY ARE POTENTIALLY INFECTIOUS. Information on handling human serum is provided in the CDC/NIH manual "Bio-safety in Microbiological and Biomedical Laboratories" (1984).

### Procedural Notes

1. All test kit components used in an assay must be of the same master lot number. Materials should not be used after the expiration date shown on the package label. Components and test specimens should be at room temperature (18°-30°C) before testing begins.
2. All calibrators, controls, and samples should be tested in duplicate simultaneously. The test samples and controls must be well mixed before use.
3. A separate disposable tip should be used for each sample to avoid cross-contamination. All pipetting steps should be performed with the utmost care and accuracy. Avoid contaminating the reagent pipette tip with the serum sample.
4. The duration of the incubation times must be the same for all wells within a run.
5. Run size should be limited to the number of samples that can be added to antibody coated wells within 5 minutes.
6. Samples should be pipetted to the bottom of the antibody-coated wells.
7. If microtiter reader is not capable of reading absorbances greater than 2.0, the color should be read after a shorter incubation time with the SUBSTRATE/CHROMOGEN, i.e., 10 minutes.

## Test Procedure

1. Place sufficient COATED WELLS in a holder to run 0.0, 0.25, 1.0, 5.0, 10 and 20  $\mu$ U/ml TSH CALIBRATORS, Quality Control Sera and patient samples in duplicate. Limit run size to the number of samples that can be pipetted in 5 minutes.
2. Pipet 50  $\mu$ l of the CALIBRATORS, Controls or Patient Sample to the corresponding COATED WELL.
3. Pipet or dispense 200  $\mu$ l of the ENZYME ANTIBODY CONJUGATE solution to all the wells and mix gently.
4. Incubate at room temperature (18°-30°C) for 45 minutes  $\pm$  5 minutes.
5. Decant or aspirate and discard liquid contents of all wells. SLAP the inverted wells on a clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
6. Fill each well with diluted WASH BUFFER. Fill the wells to overflowing, you cannot cause any carryover between the wells. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.

WARNING: WASHING THE WELLS IS OF CRITICAL IMPORTANCE. Fill the wells to overflowing, you CANNOT cause any carryover between wells. You CANNOT over wash the wells. Completely decant or aspirate all of the liquid out of the wells. SLAP the inverted wells on a FRESH clean piece of absorbent paper AFTER EACH WASH. YOU CANNOT SLAP TOO HARD, REMOVE ALL OF THE LIQUID FROM THE WELLS.

7. Repeat step 6 three more times (for a total of 4 washes).
8. Fill each well with deionized water. Fill the wells to overflowing. Decant or aspirate liquid contents of all wells. SLAP the inverted wells on a fresh clean piece of absorbent paper. Remove ALL OF THE LIQUID from the wells.
9. Pipet or dispense 100  $\mu$ l (0.1 ml) of SUBSTRATE-CHROMOGEN solution into each well.
10. Mix thoroughly and incubate 30 minutes at room temperature (18°-30°C).
11. Pipet or dispense 100  $\mu$ l (0.1 ml) of 1 N H<sub>2</sub>SO<sub>4</sub> into each well and mix thoroughly.
12. Read the absorbance of each well at 450  $\pm$  20 nm against water.

## Calculation of Results

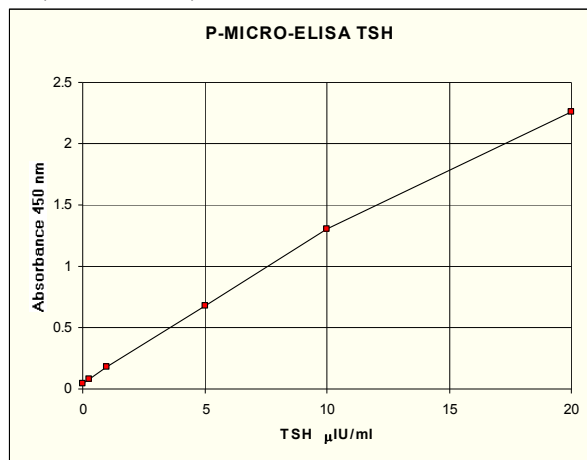
1. Calculate the mean value for each duplicate sample absorbance. Values for duplicate absorbances should be within 10% (or 0.02 absorbance units for absorbances less than 0.2).
2. Construct the standard curve by plotting the mean absorbance obtained for each TSH CALIBRATOR on the vertical (Y) axis versus the corresponding TSH concentration on the horizontal (X) axis, using rectilinear graph paper.
3. Connect the points with straight-line segments.
4. Using the mean absorbance for each sample, read the corresponding TSH concentration in  $\mu$ U/ml from the curve. Multiply the value by the dilution factor if required.



EXAMPLE DATA  
DO NOT USE IN PLACE OF CUREVE  
DETERMINED AT THE TIME OF ASSAY.

Specimen I.D.	A <sub>450</sub>	Mean A <sub>450</sub>	TSH(μIU/ml)
CALIBRATOR	0.0 μIU/ml	0.041, 0.041	0.041
CALIBRATOR	0.25 μIU/ml	0.079, 0.082	0.081
CALIBRATOR	1.0 μIU/ml	0.180, 0.179	0.180
CALIBRATOR	5.0 μIU/ml	0.694, 0.672	0.683
CALIBRATOR	10 μIU/ml	1.296, 1.313	1.304
CALIBRATOR	20 μIU/ml	2.264, 2.260	2.262

SAMPLES	A <sub>450</sub>	Mean A <sub>450</sub>	TSH(μIU/ml)
# 1 (UNKNOWN #1)	0.246, 0.253	0.250	1.6
# 2 (UNKNOWN #2)	1.139, 1.084	1.112	8.5
# 3 (UNKNOWN #3)	1.742, 1.801	1.772	14.9



The range of this assay is 0 - 20 μIU/ml. For specimen with TSH concentrations beyond the standard curve (20 μIU/ml), repeat the test by diluting the specimen with the 0 μIU/ml CALIBRATOR. To obtain the final concentration, multiply the concentration of the diluted sample by the dilution factor.

### Quality Control

Good laboratory practice requires that quality control specimen be run with each calibration curve to check the assay performance. Controls containing azide can not be used. Two controls with normal and elevated values should be used. Pooled human serum or commercially available control sera without azide are suitable. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance.

Read the absorbance of the test solutions against a water blank. If the absorbance of the 0 μIU/ml CALIBRATOR exceeds 0.100 it is an indication of careless washing and the assay must be repeated.

### Standardization

The MICRO-ELISA TSH CALIBRATORS have been standardized against the World Health Organization International Reference Preparation (2<sup>nd</sup> IRP 80/558).

### Limitations of the Procedure

As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). These samples should not be assayed with the MICRO-ELISA TSH test as erroneous results may be obtained. These conditions should be ruled out prior to clinical evaluation of test results.

The wash procedure (steps 6-8) is critical. Insufficient washing will result in poor precision and falsely elevated absorbances. The use of tap water for washing could result in a higher background absorbance.

**FINAL REACTION STABILITY:** The spectrophotometric measurement should be made within 30 minutes after the addition of the H<sub>2</sub>SO<sub>4</sub> solution.

Samples with elevated levels of TSH (up to 1,000 μIU TSH/ml) will always assay as >20 μIU TSH/ml when tested, and will not result in a "high dose hook effect". When it is necessary to measure levels of TSH greater than the 20 μIU TSH/ml CALIBRATOR, the sample should be diluted with the 0 μIU/ml CALIBRATOR and re-assayed.

### Expected Values

The MICRO-ELISA TSH test was performed on 85 randomly selected, apparently normal patient samples. The results obtained ranged from 0.3 to 7.8 μIU/ml. Statistical analysis of the results gave a geometric mean of 1.54 μIU/ml. Ninety-five percent of the individuals fell within the range of 0.4 to 4.2 μIU/ml.

The MICRO-ELISA TSH test was performed on 11 clinically diagnosed hyperthyroid patient samples. The results obtained ranged from 0.0 to 0.2 μIU/ml. 100% of the hyperthyroid individuals fell within the range of 0.0 to 0.2 μIU/ml.

The MICRO-ELISA TSH test was performed on 33 clinically diagnosed hypothyroid patient samples. The results obtained ranged from 8.0 to 156.7 μIU/ml. 100% of the hypothyroid individuals fell within the range > 8.0 μIU/ml.

Clinical Diagnosis	# Tested	Observed Values
Hyperthyroid	11	0.0 - 0.2
Euthyroid	85	0.3 - 7.8
Hypothyroid	33	8.0 - 156.7

These values are consistent with those reported in the literature. It is recommended that each laboratory determine its own normal range.



**Performance Characteristics of the Test**

**Assay Specificity**

The TSH antibodies used in the MICRO-ELISA TSH Test have no cross-reactivity with human chorionic gonadotropin (HCG) even at levels of 300,000 mIU/ml (less than 0.001% cross-reactivity). The cross-reactivity with luteinizing hormone (LH) and follicle stimulating hormone (FSH) is less than 0.001%.

**Assay Sensitivity**

The sensitivity of this assay is defined as the smallest single value that can be distinguished from the zero calibrator. This value was calculated from the mean + two standard deviations for twenty-one replicates at the zero concentration. The calculated sensitivity is <0.05 µIU/ml.

**Assay Reproducibility**

Intra-assay reproducibility was determined by measurement of 20 replicates of three serum pools in a single run.

	Mean TSH (µIU/ml)	SD	%CV
Serum A	0.87	0.09	10.3
Serum B	11.2	0.82	7.3
Serum C	15.3	0.89	5.8

The interassay reproducibility was determined by duplicate measurement of three serum pools in ten separate runs.

	Mean TSH (µIU/ml)	SD	% CV
Serum A	1.01	0.10	9.9
Serum B	10.68	0.54	5.1
Serum C	15.7	0.86	5.5

**Assay Linearity**

A study was performed diluting a serum sample containing an elevated level of TSH with the 0 µIU/ml CALIBRATOR to determine the linearity of the MICRO-ELISA TSH.

Dilution Factor	TSH µIU/ml		% of Expected Value
	Expected Value	Observed Value	
100 %	-	14.5	-
75 %	10.9	10.6	98 %
50 %	7.2	7.6	106 %
25 %	3.6	3.3	92 %
12.5 %	1.9	1.6	84 %

**Assay Recovery**

Four aliquots of human sera with a TSH concentrations of 0.02 µIU/ml were spiked with 0.75, 1.25, 5.0 and 15.0 µIU TSH/ml. The samples were assayed in duplicate.

Added TSH	TSH µIU/ml		% Recovery
	Expected Value	Measured Value	
0.0		0.02	
0.75	0.77	0.72	94 %
1.25	1.27	1.32	104 %
5.00	5.02	5.00	100 %
15.00	15.02	15.40	103 %

**Comparison to Other TSH Tests**

Correlation studies on a random group of 107 serum samples with a range of values from <0.05 – 19.6 µIU/ml, were performed using the quantitative results from the MICRO-ELISA TSH Test and an automated ELISA TSH test. The correlation coefficient of the test results was 0.957

	Slope	Y-Intercept	Correlation Coefficient
AUTOMATED n= 107	0.936	-0.038	0.957

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