

**bi-x-act**<sup>®</sup>  
of FINLAND

TSH Enzyme  
Immunoassay

CE IVD

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## Enzyme Immunoassay for the Quantitative Determination of Thyroid Stimulating Hormone (TSH) in Human Serum

### FOR IN VITRO DIAGNOSTIC USE

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

The determination of serum or plasma levels of thyroid stimulating hormone (TSH or thyrotropin) is recognized as a sensitive method in the diagnosis of primary and secondary hypothyroidism.<sup>1</sup> 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.<sup>2</sup> It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta.<sup>3</sup>

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 is responsible for maintaining the proper blood levels of these hormones.<sup>4,5</sup>

TSH and the pituitary glycoproteins: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG), have identical alpha chains.<sup>2</sup> 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 antisera. The use of a monoclonal antibody in 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.<sup>6,7,8</sup>

### **PRINCIPLE OF THE ASSAY**

The TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.<sup>9,10</sup> The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase immobilization (microtiter wells), and goat 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 a 60-minute or overnight incubation at room temperature, the solid phase is washed with water to remove unbound labeled antibodies. A solution of 3,3',5,5'-Tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

### **REAGENTS AND MATERIALS PROVIDED**

1. Antibody-Coated Wells (1 plate, 96 wells)  
Microtiter wells coated with mouse monoclonal anti-TSH.
2. Enzyme Conjugate Reagent (1 dropper bottle, 13 mL)  
Contains goat anti-TSH conjugated to horseradish peroxidase.
3. Reference Standard Set (1 mL/vial)  
Contains 0, 0.5, 2.0, 5.0, 10.0 and 25.0  $\mu$ IU/mL (WHO, 2nd IRP, 80/558) TSH in equine serum with preservatives. Lyophilized. See instructions for reconstitution under Reagent Preparation.
4. TMB Reagent (1 bottle, 11 mL)  
Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
5. Stop Solution (1N HCl) (1 bottle, 11 mL)  
Contains diluted hydrochloric acid.

### **MATERIALS REQUIRED BUT NOT PROVIDED**

1. Distilled or deionized water
2. Precision pipettes: 50  $\mu$ L, 100  $\mu$ L, 200  $\mu$ L, and 1 mL
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450nm.
5. Orbital motion microtiter well shaker, capable of shaking at a speed of 175 $\pm$ 25 RPM
6. Absorbent paper
7. Graph paper (semi-log, etc.)  
Quality control material (e.g., BioRad Lyphochek Control sera)

## **WARNINGS AND PRECAUTIONS**

1. CAUTION: Test methods are not available which can offer complete assurance that Hepatitis B virus, Human Immunodeficiency Virus (HIV/HTLV-III/LAV), or other infectious agents are absent from the reagents in this kit. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.<sup>11</sup>
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
6. Do not pipette reagents by mouth.
7. For in vitro diagnostic use.

## ***STORAGE CONDITIONS***

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

## ***INSTRUMENTATION***

A microtiter well reader with a bandwidth of 10nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

## ***SPECIMEN COLLECTION AND PREPARATION***

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. Avoid grossly hemolytic, lipemic, or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

## **REAGENT PREPARATION**

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Reconstitute each lyophilized standard with 1.0mL dH<sub>2</sub>O. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-8°C, and are stable at 2-8°C for at least 30 days.
4. Specimens expected to have a TSH concentration greater than 25µIU/mL should be diluted 1:10 with TSH-free serum (Zero calibrator).

## PROCEDURAL NOTES

1. Manual Pipetting: It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. Automated Pipetting: A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

## *ASSAY PROCEDURE*

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 $\mu$ l of standards, specimens, and controls into appropriate wells.
3. Dispense 100 $\mu$ l of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing.
5. Incubate at room temperature (18-25°C) for 60 minutes (1 hour).
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with distilled or dionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 $\mu$ l of TMB Reagent into each well. Gently mix for 5 seconds.
10. Incubate at room temperature, for 20 minutes.
11. Stop the reaction by adding 100 $\mu$ l of Stop Solution to each well.
12. Gently mix for 30 seconds. Ensure that all of the blue color changes completely to yellow.
13. Read absorbance at 450nm with a microtiter plate reader within 15 minutes.

## *CALCULATION OF RESULTS*

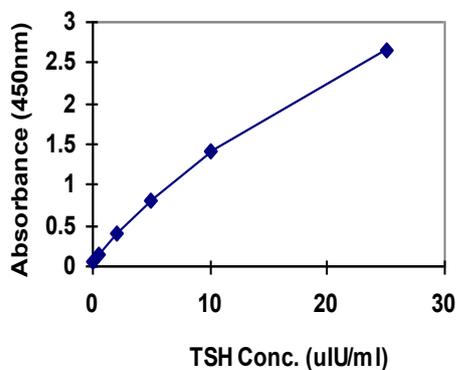
1. Calculate the mean absorbance value (OD450) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in  $\mu$ IU/mL, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of TSH in  $\mu$ IU/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

### EXAMPLE OF STANDARD CURVE

Results of a typical standard run of the assay are shown below. The standard curve is for illustration only, and should not be used to calculate unknowns. The standard curve covers a dynamic range from 0 to 25  $\mu\text{IU/mL}$ .

The absorbance (450nm) value can be varied due to incubation at different room temperatures in different laboratories.

TSH ( $\mu\text{IU/mL}$ )	Absorbance (450nm)
0	0.063
0.5	0.157
2.0	0.398
5.0	0.818
10.0	1.415



25.0	2.645
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### EXPECTED VALUES

Each laboratory should establish its own normal range based on patient population. Differences in assay technique and the use of various standards may affect results. The results provided below are based on 38 normal and 77 hyperthyroid blood specimens. The ranges were determined from the mean  $\pm$  2SD ( $\mu\text{IU/mL}$  TSH). These values may differ from other published data.

	Normal	Hyperthyroid
N	38	77
Mean TSH ( $\mu\text{IU/mL}$ )	1.44	< 0.2
Range	0.3 - 8.1	< 0.2

## NORMAL REFERENCE RANGE

The reference ranges provided below are based on Tietz.12

	TSH ( $\mu\text{U}/\text{mL}$ )
Adults	
21-54 years	0.4-4.2
55-87 years	0.5-8.9
Pregnancy	
1st Trimester	0.3-4.5
2nd Trimester	0.5-4.6
3rd Trimester	0.8-5.2

## PERFORMANCE CHARACTERISTICS

### 1. Accuracy

TSH ELISA has been compared to DPC's Immulite 2000 3rd Generation TSH test. The methods are similar in that they are both used for the quantitative determination of TSH in human serum, and both use TSH calibrated and labeled in  $\mu\text{IU}/\text{ml}$  standardized to the WHO hTSH (2nd IRP, 80/558).

A study was conducted using 39 euthyroid and 20 hypothyroid patient samples (as determined by hospital laboratory analysis). The range of samples tested was 0.4  $\mu\text{IU}/\text{mL}$  to 63  $\mu\text{IU}/\text{mL}$ . The comparison demonstrated good correlation with another commercial kit as shown below:

N = 59

Correlation coefficient = 0.994

Slope = 1.027

Intercept = 0.787

TSH ELISA KIT Mean = 8.39  $\mu\text{IU}/\text{mL}$

DPC Mean = 9.41  $\mu\text{IU}/\text{ml}$

Another 77 hyperthyroid patient samples also correlated well with the DPC test kit:

	# Samples with [TSH] $\leq$ 0.2 $\mu\text{IU}/\text{mL}$
DPC Kit	N = 77
TSH ELISA Kit	N = 77

### 2. Sensitivity

At TSH concentrations of 0.1  $\mu\text{IU}/\text{mL}$  and 0.2  $\mu\text{IU}/\text{mL}$ , the interassay CVs were determined to be 11.4% and 7.9%, respectively.

### 3. Precision

#### a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3	4
Number of Replicates	28	26	26	26
Mean TSH ( $\mu$ IU/ml)	0.62	1.51	15.48	26.14
Standard Deviation	0.03	0.09	0.39	0.86
Coefficient of Variation (%)	4.6%	5.7%	2.5%	3.3%

#### b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of four different serum samples over a series of individually calibrated assays as shown below:

Serum Sample	1	2	3	4
Number of Replicates	30	24	24	24
Mean TSH ( $\mu$ IU/ml)	0.64	1.46	15.38	25.26
Standard Deviation	0.05	0.10	0.87	1.75
Coefficient of Variation (%)	7.6%	7.1%	5.7%	8.9%

### 4. Recovery and Linearity Studies

#### a. Recovery

Various patient serum samples of known TSH levels were combined and assayed in duplicate. The mean recovery was 98.9%.

Expected Concentration ( $\mu$ IU/ml)	Observed Concentration ( $\mu$ /ml)	% Recovery
23.94	22.92	95.7%
11.75	11.01	93.6%
5.46	5.53	101.3%
2.76	3.04	110.1%
1.48	1.39	93.9%

### b. Linearity

Two patient samples were serially diluted with zero standard to determine linearity. The mean recovery was 94.6%.

#	Dilution	Expected Conc. (μIU/ml)	Observed Conc. (μIU/ml)	% Expected
1	Undiluted	24.42	48.39	91.9%
	1:2	11.21	22.43	93.2%
	1:4	5.60	10.45	91.6%
	1:8	2.80	5.13	102.5%
	1:16	1.40	2.87	92.9%
	1:32		1.30	
				Average = 94.4%
2	Undiluted	18.21	36.42	99.4%
	1:2	9.10	18.10	93.2%
	1:4	4.55	8.48	94.1%
	1:8	2.27	4.28	97.8%
	1:16	1.13	2.22	89.4%
	1:32		1.01	
				Average = 94.8%

### 6. Specificity

The following hormones were tested for cross-reactivity:

HORMONE TESTED	CONCENTRATION	PRODUCed INTENSITY EQUIVALENT to TSH (μIU/mL)
HCG - (WHO 2nd IS 61/6)	100 mIU/mL	0
	600 mIU/mL	0
	3,500 mIU/mL	0
	10,000 mIU/mL	0
	200,000 mIU/mL	0
FSH - (WHO 2nd IRP-HMG)	20 mIU/mL	0
	100 mIU/mL	0
	200 mIU/mL	<0.2
LH - (WHO 1st IRP 68/40)	75 mIU/mL	0
	150 mIU/mL	0
	300 mIU/mL	<0.2
Prolactin - (WHO 1st IRP 75/504)	10 ng/mL	0
	50 ng/mL	0
	200 ng/mL	<0.2
hGH - (WHO 1st IRP 65/217)	10 ng/mL	0
	50 ng/mL	0
	200 ng/mL	<0.2

## 5. Hook Effect

No hook effect is observed in this assay at TSH concentrations up to 1,000  $\mu\text{IU/mL}$ .

### Quality Control

Good laboratory practice requires that low, medium and high quality control specimens (controls) 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.

### Standardization

The TSH Reference Standards are calibrated against the World Health Organization's Second International Reference Preparation of hTSH 2nd IRP-80/558).

### Limitations of the Procedure

1. 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.
2. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. The TSH assay has not been tested on newborns, and is not for use in screening newborns.

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