

TSH ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the
Quantitative Determination of
Thyroid Stimulating Hormone (TSH)
Concentration in Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-TSH-96

PISHTAZ TEB DIAGNOSTICS

Introduction

The determination of serum or plasma levels of thyroid stimulating hormone (TSH) is recognized as a sensitive method in the diagnosis of primary and secondary hypothyroidism. TSH is secreted by the anterior lobe of the pituitary gland and induces the production and release of thyroxine and triiodothyronine from the thyroid gland. It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta. 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 hormones. When there is a high level of thyroid hormones 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 are decreased thyroid hormones in the blood. This process is known as a negative feedback mechanism and is responsible for maintaining the proper blood levels of these hormones. TSH and the pituitary glycoproteins: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG), have identical alpha chains. The beta chain is distinct but does contain identical amino acid sequences, which can cause considerable cross-reactivity with some polyclonal TSH antisera. The use of a

monoclonal antibody in this TSH EIA 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".

Test Principle

The TSH ELISA test is based on the principle of a sandwich enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. A mouse monoclonal anti-TSH antibody is used for solid phase immobilization and another anti TSH monoclonal antibody conjugated to HRP. 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 1 hour incubation at room temperature, the wells are washed with washing solution to remove unbound labeled antibodies. A solution of TMB is then added and incubated for 15 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

Materials provided with the kit:

1. Antibody coated Wells(1 plate , 96 wells):
Microtiter wells coated with monoclonal anti human TSH.
2. Enzyme conjugate (1 vial , 12 ml):
Monoclonal anti human TSH labeled with HRP in buffer containing protein as stabilizer and Thiomerosal as preservative, ready to use.
3. Standard Set (1 ml / 6 vials): Contain 0.0 (2 ml), 0.5, 2.5, 5, 15, and 30 mIU/L of TSH calibrated against WHO 80/558 2nd IRP in buffer containing protein as stabilizer and Thiomerosal as preservative, ready to use.
4. Control serum (1 vial, 1 ml): Contains certain amount of human TSH in buffer containing protein as stabilizer and Thiomerosal as preservative, ready to use.
5. Chromogen Substrate Reagent (1 vial, 12ml): Contains Tetra-methyl benzidine and hydrogen peroxide, ready to use.

6. Wash solution (1 vial, 50 ml, concentrated 20x): Contains Phosphate buffer solution with 0.05% Tween 20.
7. Stop solution (1 vial, 12 ml) : Contains 1M Hydrochloric acid.
8. Cardboard sealer.

Materials required but not provided

1. ELISA reader with 450 nm filter.
2. Precision micropipettes: 50 and 100 μ l.
3. Distilled water.
4. Disposable pipette tips.
5. Absorbent paper or paper towel.
6. Graph paper.

General Information

1. Do not mix kit reagents from different batch/lot numbers. All kit components must be used only in their original kit.
2. All reagents obtained from human sources are negative for HBs Ag, anti HCV and anti HIV antibodies. To prevent risk of contamination, use personal protective equipments like gloves, lab coats, etc. and avoid direct contact with reagents.

Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep Un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.

Specimen Collection and Preparation

The kit is for use with serum or plasma. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return to room temperature before testing. If prolonged storage is required, samples should be stored at

-20 °C (maximum 30 days). Avoid freeze-thaw of specimen during storage.

Reagents Preparation

1. All reagents should be allowed to reach room temperature (22-28°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.

Assay Procedure

1. Secure the desired number of microplate wells in the holder and keep the remaining with desiccants in tightly closed original bag.
2. Dispense 50 μ l of each standard, serum control and specimen into appropriate wells.
3. Add 100 μ l of Anti-TSH-HRP conjugate to the wells.
4. Cover the microtiter wells with cardboard sealer tightly. Leave them for 60 minutes at room temperature (22-28°C).
5. Remove the incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each time with 300 μ l of working wash solution). Strike the wells sharply onto absorbent paper or paper towel to remove all residual wash solution droplets.
6. Dispense 100 μ l of chromogenic substrate solution to the microplate wells.
7. Incubate the microplate wells at room temperature and dark for 15 minutes; to develop color.
8. Add 100 μ l of stop solution to the wells to stop reaction.
9. Read absorbance at 450 nm by ELISA reader (Use 630 nm filter as reference filter if it's available)

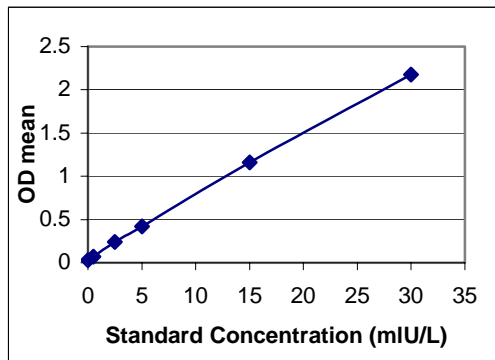
Results Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm. (Use 630 nm filter as reference filter if it's

- available)
- Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in mIU/L on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
 - Use the mean absorbance value for each sample, determine the corresponding concentration of TSH in mIU/L from the standard curve.

Example of Standard curve

Standards (mIU/L)	Absorbance
0.0	0.031
0.5	0.071
2.5	0.241
5.0	0.422
15.0	1.160
30.0	2.175



Note: The standard curve shown in above example is for the purpose of illustration only and should not be used to calculate unknowns. Users should obtain their own data and standard curve.

Expected Values

The normal value for TSH was determined by repeated ELISA tests on sera of normal population and results are shown below. However each medical laboratory must determine own

normal references.

Reference interval in adults mIU/L	Mean mIU/L
0.32-5.2	1.8

Performance Characteristics

1. Minimum Detection Limit

Based on mean absorbance of zero standard + 3SD, the minimum concentration of TSH which is detected by this assay is 0.1 mIU/L.

2. Test Precision

To determine intra-assay and inter-assay of this kit, replicate tests were done on four different serum samples with different TSH concentrations. Results are shown in table 1 and 2:

Table 1 (Intra-assay)

Specimen No.	No.of tests performed	Means mIU/L	SD mIU/L	CV%
1	16	2.5	0.16	6.4
2	16	4.35	0.17	3.9
3	16	11.7	0.53	4.5

Table 2 (Inter-assay)

Specimen No.	No.of tests performed	Means mIU/L	SD mIU/L	CV%
1	20	0.25	0.02	8.0
2	20	4.4	0.31	7.0
3	20	12.1	1.02	8.42

Each test has been run in duplicate

3. Test Recovery

To assess test recovery, certain amount of TSH was added into 4 different sera with known concentration of TSH and the sera were tested by TSH ELISA test. The recovery was determined for each serum and results are shown below:

Table 3: Test recovery

No.	TSH level mIU/L	TSH added mIU/L	Exp. value mIU/L	Obs. Value mIU/L	Rec. (%)
1	0.6	0.5	0.55	0.6	109
1	0.6	5	2.8	2.6	93
1	0.6	15	7.8	7.5	96
2	1.8	0.5	1.1	1.1	100
2	1.8	5	3.4	3.6	105
2	1.8	15	8.4	8.8	105
3	2.9	0.5	1.7	1.6	95
3	2.9	5	3.9	4.1	105
3	2.9	15	8.9	9	101
4	5.0	0.5	2.7	2.5	92
4	5.0	5	5	4.8	96
4	5.0	15	10	10.5	105

Exp.: Expected , Obs.: Observed,
 Rec.: Recovery

4. Test Linearity

To determine test linearity four different serum samples were diluted sequentially by zero standard. Then the sera were tested by TSH ELISA test. The results and serum recovery were determined considering dilution factor. The results are shown below:

Table 4: Test linearity

No.	TSH undiluted specimen	Recovery (%)			
		1:2	1:4	1:8	1:16
1	2.7	100	98	102	96
2	8.4	99	103	99	96
3	16.7	99	94	104	105
4	27.8	97	105	94	85

5. Test Specificity

To verify cross reaction of kit with other closely related hormones, the following hormones were tested for cross-reactivity (table 5):

Table 5 (Specificity test result, cross reaction)

Analyte name	Concentration (IU/L)	Cross reaction (mIU/L)
hFSH	1000	<0.1
	100	<0.1
	10	<0.1
hLH	1000	<0.1
	100	<0.1
	10	<0.1
hCG	100000	<0.1
	10000	<0.1
	1000	<0.1

6. Correlation test

Pishtaz Teb Diagnostics TSH ELISA Kit has been compared to the market leader IRMA Kit. A study conducted using 128 euthyroid, hyperthyroid and hypothyroid sample ranging in value 0.1 to 30 mIU/L. The comparison demonstrated good correlation with another commercial kit as shown below:

N = 128

Correlation Coefficient : 0.99

Slope = 0.971, Intercept = 0.117

Mean test = 3.64, Mean reference = 3.65

7. Hook effect




The TSH assay was done on sera with high concentration of TSH (up to 500 mIU/L) and no "hook effect" was seen.

References

1. Cobb W.E., Lamberton R.P, Jackson I.M.D. (1984) Use of a rapid, sensitive immunoradiometric assay for thyrotropin to distinguish normal from hyperthyroid subjects. *Clin.Chem.* 30:1558-1560.
2. Helenius T., Tikanoja S. (1986) A sensitive and practical immunoradiometric assay of thyrotropin. *Clin. Chem.* 32:514-518.
3. Woodhead J.S., Weeks I., (1985) Circulating thyrotropin as an index of thyroid function. *Ann. Clin. Biochem.* 22:455-459.
4. Lamberg B.A., Helenius T., Liewendahl K. (1986) Assessment of thyroxine suppression in thyroid carcinoma patients with a sensitive immunoradiometric TSH assay. *Clin. Endocrinol.* 25:259-263.

TSH Test Procedure

Step 1




	Standard	Control	Sample
Reagents			
Standard	50 µl	None	None
Control	None	50 µl	None
Sample	None	None	50 µl
Anti TSH-HRP conjugate	100 µl	100 µl	100 µl

Gently shake wells for 15 seconds to mix contents of the wells. Cover microplate wells with cardboard sealer and leave them for 60 minutes at room temperature.






Step 2

Remove cover and tap plate contents into a waste container. Wash the microplate wells for 5 times according to test manual.

Chromogen-substrate solution	100 µl 	100 µl 	100 µl 
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Incubate the microplate wells for 15 minutes at room temperature and dark.

Step 3

Stop Solution	100 µl 	100 µl 	100 µl 
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Read well absorbance at 450nm (and 630nm as reference filter) by ELISA reader and calculate the results.