

T4 ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the
Quantitative Determination of Thyroxine (T4)
Concentration in Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-T4-96

PISHTAZ TEB DIAGNOSTICS

Introduction

Thyroxine hormone is synthesized in thyroid gland which is most important component of endocrine system. More than 99% of thyroxine (T4) in blood is bound to carrier proteins; thyroxine-binding globulin (TBG), albumin and prealbumin. The total thyroxine content of blood (total T4) is important in evaluating thyroid disorders. In hyperthyroidism like those with Grave's disease, T4 level is increased and in hypothyroidism like myxedema, T4 level is decreased.

Test Principle

The test principal is based on competitive ELISA technique. In this technique wells are coated by certain amount of anti-T4 monoclonal antibody, (Anti-T4 mAb). Then patient serum, assay buffer and a constant amount of T4-HRP conjugate are added to microtiter wells. During the incubation, the T4 and conjugated T4 compete for the limited binding sites on the wells. The wells are completely washed to remove unbound T4. A solution of TMB-substrate is the added and incubated, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm, the intensity of the color formed is proportional to the amount of enzyme present and in inversely related to unlabeled T4 in the sample. By reference to a series of T4 standards assayed in

the same way, the concentration of T4 in the unknown sample is quantified.

Materials Provided with Test Kit

1. Antibody coated wells (1 plate, 96 wells): Microtiter wells coated with monoclonal anti Thyroxin.
2. Enzyme conjugate (1vial, 6 ml): Thyroxin labeled with HRP in buffer containing protein as stabilizer and Thiomersal as preservative, ready to use.
3. Standards set (0.5 ml / vial): Contains 0.0 (1 ml), 2, 4, 8, 12, and 20 $\mu\text{g}/\text{dl}$ of Thyroxin in buffer containing protein as stabilizer and Thiomersal as preservative, ready to use.
4. Control serum (1 vial, 0.5 ml): Contains certain amount of Thyroxin in buffer containing protein as stabilizer and Thiomersal as preservative, ready to use.
5. Assay buffer (1 vials, 6 ml): Contains phosphate buffer solution with protein as stabilizer and Cathon CG as preservative, ready to use.
6. Chromogen substrate reagent (1vial, 12 ml): Contains Tetra Methyl Benzedine and hydrogen peroxide, ready to use.
7. Wash solution (1 vials, 50 ml concentrated 20x): Contains phosphate buffer solution with 0.05 % Tween 20.
8. Stop solution (1 vial, 12 ml): Contains 1 molar Hydrochloric acid.
9. Cardboard sealer.

Materials/Equipments required but not provided with Test Kit

1. ELISA reader.
2. Precision pipettes: 25 μl ,50 μl and 100 μl micropipettes.
3. Distilled water.
4. Disposable pipette tips.
5. Vortex mixer or equivalent.
6. Absorbent paper.
7. 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 $2-8^{\circ}\text{C}$ 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 prolong 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 ($22-28^{\circ}\text{C}$) 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 coated wells in the holder and keep the remaining with desiccants in tightly closed sealed bag.
2. Dispense $25\ \mu\text{l}$ of each standard, serum control and specimen into appropriate wells.
3. Add $50\ \mu\text{l}$ of assay buffer to the microplate wells.
4. Add $50\ \mu\text{l}$ of T4-HRP conjugate into the wells.
5. Shake microplate wells gently for 15 seconds to mix well contents. Cover the microplate wells with cardboard sealer provided with kit and incubate for 1 hour at room temperature and dark.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Wash the microplate wells 5 times (each time with $300\ \mu\text{l}$ of working wash solution). Each washing step consists of three stages of rinse, gentle shake and pour off wash solution into a waste container. Strike the wells sharply onto absorbent paper to remove residual wash droplets.
8. Add $100\ \mu\text{l}$ of chromogenic substrate (TMB) into the wells.
9. Incubate the wells for 15 minutes at room temperature and dark.
10. Add $100\ \mu\text{l}$ of stop solution into the microplate wells to stop enzymatic reaction.
11. Read OD at 450 nm with ELISA reader within 30 minutes. (Use 630 nm filter as reference filter if it's available)

Results Calculation

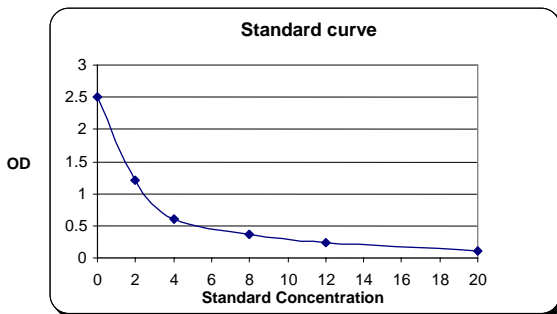
1. Measure absorbance of standards and samples at 450 nm and calculate mean of duplicate specimens. (Use 630 nm filter as reference filter if it's available).
2. Construct a point to point standard curve

by plotting the mean absorbance obtained for each reference standard against its concentration in $\mu\text{g/dl}$ on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

- Using the mean absorbance value for each sample, determine the corresponding concentration of T4 ($\mu\text{g/dl}$) from the standard curve.

Example of Standard curve

Standards ($\mu\text{g/dl}$)	Absorbance
0	2.5
2	1.21
4	0.61
8	0.37
12	0.24
20	0.11



Note: this standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must provide its own data and standard curve.

Expected Values

The normal value for T4 is determined by repeated T4 ELISA test on sera of people from normal population. However, each medical laboratory must determine its own normal references.

Mean in adults $\mu\text{g/dl}$	Normal range in adults $\mu\text{g/dl}$
8.6	4.7-12.5

$$\mu\text{g/dl} \times 12.87 = \text{nmol/L}$$

$$\text{nmol/L} \times 0.078 = \mu\text{g/dl}$$

Performance Characteristics

1. Minimum Detection Limit

Based on mean absorbance of zero standard + 3SD, the minimum concentration of T4 which is detected by this assay is 0.4 $\mu\text{g/dl}$.

2. Test Precision

To determine intra-assay and inter-assay of this kit, replicate tests were performed on 3 different serum samples with different T4 concentrations. Results are shown in table 1 and 2:

Table 1 (Intra-assay)

Specimen No.	Tests	Mean $\mu\text{g/dl}$	SD $\mu\text{g/dl}$	CV%
1	24	3.6	0.18	5.0
2	24	9.5	0.55	5.7
3	24	16	0.58	3.6

Table 2 (Inter-assay)

Specimen No.	Tests*	Means $\mu\text{g/dl}$	SD $\mu\text{g/dl}$	CV%
1	10	3.8	0.21	5.5
2	10	9.9	0.76	7.6
3	10	15.3	0.68	4.4

*Each test has been run in duplicate

3. Test Recovery

To assess test recovery, certain amount of T4 was added into 4 different sera with known concentration of T4 and the serums were tested by T4 ELISA test. The recovery was determined for each serum and results are shown in table 3:

Table 3: Test recovery

No.	T4 level µg/dl	T4 added µg/dl	Exp. value µg/dl	Observed value µg/dl	Rec.%
1	2.5	2	2.25	2.4	107
1	2.5	8	5.2	5.1	98
1	2.5	20	11.2	12	107
2	4	2	3	2.8	93
2	4	8	6	6.3	105
2	4	20	12	12	100
3	7	2	4.5	4.2	93
3	7	8	7.5	7.1	95
3	7	20	13.5	13.9	103
4	11	2	6.5	7.1	109
4	11	8	9.5	9	95
4	11	20	15.5	16.5	109

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

4. Test Specificity

The following hormones were tested for cross-reactivity:

Table 4 (Specificity of test result, Cross reaction)

Analyte name	Concentration (nmol/l)	Apparent T4 level (µg/dl)
3,5-Diiodothyronine	1000	< 0.4
3,3',5-Triiodothyronine (T3)	100	< 0.4
3,3',5'-Triiodothyronine (rT3)	100	< 0.4
3,3',5-Triiodothyroacetic acid	100	< 0.4
3,3',5-Triiodothyropropionic acid	100	< 0.4

5. Test Linearity

To determine test linearity four different serum samples were diluted sequentially by zero standard. Then the sera were tested by PishtazTeb T4 ELISA test. The results and serum recovery were determined considering dilution factor. The results are shown below:




Table 5: Test linearity

No.	T4 undiluted specimen	Recovery (%)			
		1:2	1:4	1:8	1:16
1	8.5	95	106	91	-
2	12	94	91	109	-
3	21	96	90	92	91
4	28	90	94	109	107

References

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- Cavalieri RR., Rapoport B. (1977) Impaired peripheral conversion of thyroxine to triiodothyronine. *Ann. Rev. Med.* 28:57-65
- Spector DA *et al.* (1976) Thyroid function and metabolic state in chronic renal failure *Ann. Int. Med.* 85:724-730.
- Burr WA *et al* (1975) Serum triiodothyronine and reverse triiodothyronine concentration after surgical operation. *Lancet* II 1277-1279.




T4 Test Procedure
Step 1

Reagents	Standard	Control	Sample
			
Standards	25 µl	--	--
Control Serum	--	25 µl	--
Sample	--	--	25 µl
Assay buffer	50 µl	50 µl	50 µl
T4-HRP conjugate	50 µl	50 µl	50 µl

Shake wells gently for 15 seconds to mix reagents. Cover wells with cardboard sealer. Incubate for 1 hour at room temperature and dark.





Step 2

Remove plate cover and discard contents of the wells. Wash the microplate wells for 5 times according to test manual.

Chromogen-substrate solution	100 µl	100 µl	100 µl
			

Incubate wells for 15 minutes at room temperature in dark.


Step 3

Stop Solution	100 µl	100 µl	100 µl
			

Measure well absorbance at 450 nm (and 630 nm as reference filter) by ELISA reader and calculate results.