

## FREE T4 ELISA Kit

48 Tests Kit

Enzyme Immunoassay for the

Quantitative Determination of Free Thyroxine (FREE T4)

Concentration in Human Serum/Plasma

(For In Vitro Diagnostic Use Only)

Catalogue No. PT-FT4-48

**PISHTAZ TEB DIAGNOSTICS**

### Introduction

Thyroxine hormone is synthesized in thyroid gland which is most important component of endocrine system. Over than 99% of thyroxine (T4) circulated in blood is bound to carrier proteins; thyroxine-binding globulin (TBG), albumin and prealbumin. However, only the free (unbound) portion of T4 is responsible for the biological action. Furthermore, the concentrations of the carrier proteins are altered in many clinical conditions, such as pregnancy. In normal thyroid function as the concentrations of the carrier proteins alter, the total T4 level changes whereas the free T4 concentration remains constant. Thus, measurements of free T4 concentration correlate more reliably with clinical status than total T4 levels. The increase in total T4 levels associated with pregnancy, oral contraceptives and estrogen therapy result in higher total T4 levels while the free T4 concentration remains basically unchanged.

### Test Principle

The test principle is based on competitive ELISA technique. In this technique wells are coated by certain amount of anti-T4 monoclonal antibody (Anti- T4 mAb). A measured amount of patient serum, standards and a constant amount of T4-HRP conjugate are added to the microtiter wells. During the incubation, the free T4 and

conjugated T4 compete for the limited binding sites on the wells. The wells are completely washed to remove unbound free T4. A solution of TMB- substrate is then 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 is inversely related to the amount of unlabeled free T4 in the sample.

### Materials Provided with Test Kit

1. Antibody coated wells (1 plate, 48 wells): Microtiter wells coated with monoclonal anti Thyroxin.
2. Enzyme conjugate (1vial, 3 ml): Thyroxin labeled with HRP in buffer containing protein as stabilizer and Thiomerosal as preservative, ready to use.
3. Standard Set (0.5 ml / vial): Contains 0.0, 0.2, 0.8, 2, 4, and 8 ng/dl of Free Thyroxin in buffer containing protein as stabilizer and thiomerosal as preservative, ready to use.
4. Control Serum (1 vial, 0.5 ml): Contains certain amount of Free Thyroxin in buffer containing protein as stabilizer and thiomerosal as Preservative, ready to use.
5. Assay buffer (1 vials, 3ml): Contains Phosphate buffer solution with protein as stabilizer and Kathon CG as preservative, ready to use.
6. Chromogen substrate reagent (1 vial, 6 ml): Contains Tetra Methyl Benzidine and hydrogen peroxide, ready to use.
7. Wash solution (1 vials, 25 ml concentrated 20x): Contains Phosphate buffer solution with 0.05 % Tween 20.
8. Stop solution (1 vial, 6 ml): Contain Hydrochloric acid (1M).
9. Cardboard sealer.

### Materials/Equipments required but not provided with Test Kit

1. ELISA reader

1



2. Precision pipettes: 25µl, 50µl and 100µl 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, HCV and 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 returns 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°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 µl of each standard, serum control and specimen into appropriate wells.
3. Add 50 µl of Assay buffer into the wells.
4. Add 50 µl 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.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Wash the microplate wells 5 times (each time with 300 µl 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 µl of chromogenic substrate solution into the wells.
9. Incubate the wells for 15 minutes at room temperature and dark.
10. Add 100 µl of stop solution into the microplate wells to stop enzyme 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

2

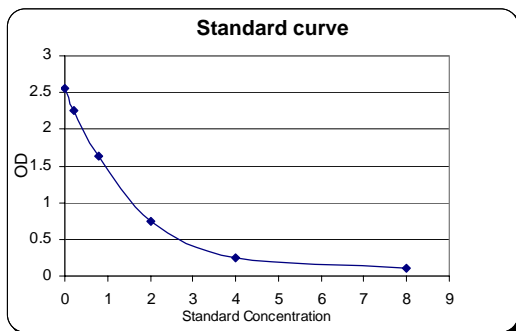


obtained for each reference standard against its concentration in ng/dl on linear graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

- Using the mean absorbance value for each sample, determine the corresponding concentration of FREE T4 in ng/dl from the standard curve.

### Example of Standard curve

Standards (ng /dl)	Absorbance
0.0	2.55
0.2	2.25
0.8	1.63
2.0	0.75
4.0	0.25
8.0	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 FREE T4 is determined by repeated FREE T4 ELISA test on sera of people from normal population. However, each medical laboratory must determine own normal references.

Normal range in adults ng /dl
0.7-1.8

## Performance Characteristics

### 1. Minimum Detection Limit

Based on mean absorbance of zero standards minus 3SD, the minimum concentration of FREE T4 which is detected by this assay is 0.1 ng /dl.

### 2. Test Precision

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

Table 1: Intra-assay

Specimen No.	Tests	Mean ng /dl	SD ng/dl	CV%
1	24	0.5	0.03	6.0
2	24	1.5	0.05	3.3
3	24	7.4	0.26	3.5

Table 2 : Inter-assay

Specimen No.	Tests*	Means ng /dl	SD ng /dl	CV%
1	10	0.56	0.04	7.0
2	10	1.55	0.06	3.8
3	10	7.67	0.35	4.5

\*Each test has been run in duplicate

### 3. Test Recovery

To assess test recovery, certain amount of FRET4 was added into 4 different serums with known concentration of FREE T4 and the sera were tested by Pishtaz teb FREE T4 ELISA test. The recovery was determined for each serum and

3



results are shown below:

Table 3: Test recovery

No.	FREE T4 level ng /dl	FREE T4 added ng /dl	Exp. value ng /dl	Observed value ng /dl	Rec .%
1	0.53	0.2	0.36	0.34	95
1	0.53	0.8	0.66	0.65	98
1	0.53	2	1.26	1.15	91
2	0.95	0.2	0.57	0.6	105
2	0.95	0.8	0.87	0.8	92
2	0.95	2	1.47	1.5	102
3	1.5	0.2	0.85	0.83	97
3	1.5	0.8	1.15	1.19	103
3	1.5	2	1.75	1.86	106
4	3.1	0.2	1.65	1.6	96
4	3.1	0.8	1.95	1.81	93
4	3.1	2	2.55	2.58	101

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

#### 4. Test Linearity

To verify test linearity, 3 different serum samples with known free T4 concentrations were diluted sequentially by zero standard. Then the sera were tested by Pishtaz teb Free T4 ELISA test. The results and serum recoveries were determined considering dilution factor.

Table 4: Test linearity

No.	Free T4 (ng/dl) undiluted specimen	Recovery (%)			
		1:2	1:4	1:8	1:16
1	7.8	107	103	102	98
2	6.5	95	101	99	107
3	3.4	92	109	91	104

#### 5. Test Specificity

The following hormones were tested for cross-reactivity:

Table 5 : Specificity of test result, and Cross reaction.




Analyte name	Concentration (nmol/l)	Apparent FT4 level (ng/dl)
3,5-Diiodothyronine	1000	< 0.1
3,3',5-Triiodothyronine (T3)	100	< 0.1
3,3',5'-Triiodothyronine (rT3)	100	< 0.1
3,3',5-Triiodothyroacetic acid	100	< 0.1
3,3',5-Triiodothyropropionic acid	100	< 0.1

#### References

1. Tietz N.W, Fundamentals of clinical chemistry, 2nd Ed, Pg, 602 Saunders press, Philadelphia, 1976
2. Barker, S.B. – Determination of protein bound iodine journal biological chemistry, 173, 175, (1948)
3. Young, D.S, Pestaner, L.C and Gilberman, U young effects of drugs on clinical laboratory tests clinical chemistry, 21, 3660, (1975)
4. Sati, c. , chattror, A.J. , Watts, N. in fundamentals of clinical chemistry. Ed, Tietz, N.W. 3rd Edition, pg. 586, Saunders press Phila. 1987
5. Liewendhal K. (1990) Assessment of thyroid status by laboratory methods: development and prepectives. *Scan J. clin. Invest.* (Suppl. 201) 83-92
6. Cavalieri RR., Rapoport B. (1977) Impaired peripheral conversion of thyroxine to triiodothyronine. *Ann. Rev. Med.* 28:57-65
7. Spector DA *et al* . Thyroid function and metabolic state in chronic renal failure *Ann. Int. Med.* 85:724-730.
8. Burr WA *et al* (1975) Serum triiodothyronine and reverse triiodothyronine concentration after surgical operation. *Lancet* II






**FREE T4 Test Procedure**
**Step 1**

Reagents	Standard	Control	Sample
			
Standards	25 µl	--	--
Serum Control	--	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.

**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

Stop Solution	100 µl	100 µl	100 µl
			

**Step 3**

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