

## FREE T3 ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the  
Quantitative Determination of  
FREE T3 (FT3)Hormone Concentration  
In Human Serum/Plasma  
(For In Vitro Diagnostic Use Only)  
Catalogue No. PT-FT3-96

**PISHTAZ TEB DIAGNOSTICS**

### Introduction

3,5,3' triiodothyronine (T3) hormone secreted by thyroid gland and circulates in the bloodstream, mostly (99.7%) bound to the plasma proteins, thyroxine binding globulin (TBG), prealbumin and albumin. The remaining (0.3%) is free(FT3), unbound and its metabolic potency is much greater. T3 hormone regulates cell metabolism and body growth and its level is good indicator of thyroid disease state and body metabolism. Alteration of plasma proteins in clinical conditions such as pregnancy and drugs usage like contraceptive pills, estrogen therapy, phenytoins, androgens lead to falsely increase in total T3 but not in free T3 level. Therefore assessing the FT3 is a reliable diagnostic tool for estimation of thyroid disease.

### Test Principle

The test principle is based on competitive ELISA technique. In this technique wells are coated by certain amount of anti-T3 monoclonal antibody (mAb Anti-T3). A measured amount of patient serum and standards are added to the microtiter wells, after the first incubation a constant amount of T3 conjugated with horseradish peroxidase is added. During the second incubation, the unconjugated and conjugated T3 compete for the limited binding sites on the wells.

The wells are completely washed to remove unbound FT3. A solution of TMB- substrate is then added and incubated which results 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 FT3 in the sample. By reference to a series of FT3 standards assayed in the same way, the concentration of FT3 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 Triiodothyronine.
2. Enzyme conjugate (1vial, 6 ml): Triiodothyronine labeled with HRP in buffer Containing protein as stabilizer and Thiomerosal as preservative, ready to use.
3. Standard set (1 ml / vial): Contains 0.0, 2.0, 4.0, 8.0, 12, and 20 pg/ml of FT3 in buffer containing protein as stabilizer and Thiomerosal as preservative, ready to use.
4. Control serum (1 vial, 1 ml): Contains certain amount of FT3 in buffer containing protein as stabilizer and Thiomerosal as preservative, ready to use.
5. Assay buffer (1 vials, 6ml): Contains Phosphate buffer ssolution with protein as stabilizer and Kathon CG as preservative, ready to use.
6. Chromogen substrate reagent (1 vial, 12 ml): Contains Tetra Methyl Benzidine (TMB) 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 Hydrochloric acid (1M).
9. Cardboard sealer.

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

1. ELISA reader.
2. Precision pipettes: 50 and 100  $\mu$ l 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 lot numbers. All kit components must be used only in 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<sup>o</sup> 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°C) before use.
2. Working wash solution: dilute concentrated wash solution 1:20 with distilled water before use.

### **Assay Procedure**

1. Use required number of wells and keep the remaining with desiccants in tightly closed sealed bag.
2. Add 50 $\mu$ l of each standard, serum control and specimen into appropriate wells.
3. Add 50  $\mu$ l of assay buffer into the wells.
4. Shake the microplate gently for 15 seconds to mix reagents and cover the wells with provided cardboard sealer.
5. Incubate the wells for 30 minutes at room temperature (22-28°C).
6. Add 50  $\mu$ l of T3-HRP conjugate into the wells.
7. Shake microplate wells gently for 15 seconds to mix reagents. Cover it again with cardboard sealer and incubate for 30 min. at room temperature.
8. Wash the microplate wells 5 times (each time with 300  $\mu$ l of working wash solution). Shake wells gently, pour off wash solution into a waste container and strike the wells sharply onto absorbent paper to remove residual wash droplets.
9. Add 100  $\mu$ l of chromogen-substrate solution into the wells.
10. Leave the microplate for 15 minutes at room temperature and dark.
11. Add 100  $\mu$ l of stop solution into the wells to stop enzymatic reaction.
12. Read OD at 450 nm with ELISA reader within 30 minutes (Use 630 nm filter as reference filter if it's available).

### **Results Calculation**

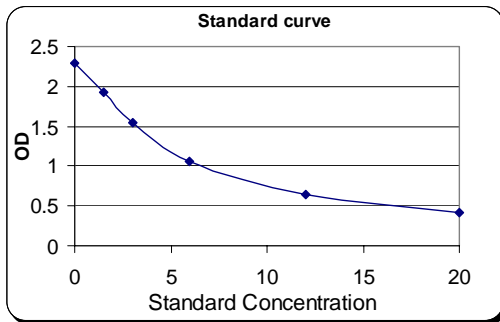
1. Measure absorbance of standards and samples at 450nm (and 630nm as reference filter) and calculate mean of duplicate specimens.
2. Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in



pg/ml 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 FT3 in pg/ml from the standard curve.

Standards Pg/ml	Absorbance
0	2.3
2.0	1.93
4	1.55
8	1.06
12	0.65
20	0.41



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 FT3 was determined by repeated ELISA tests on sera of normal people and results shown below, but it is highly recommended that each medical laboratory use its own normal references.

Reference Interval (pg/ml)	Mean (pg/ml)
1.9-4.3	3.1

## Performance Characteristics

### 1. Minimum Detection Limit

Based on mean absorbance of zero standard minus 3SD, the minimum concentration of FT3 which is detected by kit is 0.5 pg/ml.

### 2. Test Precision

To determine intra-assay and inter-assay of kit, multiple tests were performed on three different sera with different FT3 concentrations. Results are shown in table 1 and 2:

Table 1: Intra-assay

Specimen No.	Number of tests	Means pg/ml	SD pg/ml	CV%
1	24	1.25	0.05	4.0
2	24	2.72	0.13	4.7
3	24	7.75	0.31	4.0

Table 2: Inter-assay

Specimen No.	Number of tests	Means pg/ml	SD pg/ml	CV%
1	10	1.43	0.08	5.6
2	10	2.89	0.23	7.9
3	10	8.77	0.48	5.5

### 3. Test Specificity

To determine kit specificity, sera with different concentrations of L-Thyroxine, Diiodothyronine, Diiodothyrosine, Iodothyrosine, Phenylbutazone and sodium Salicylate were tested. Results are shown in table 3.

Table 3: Specificity and cross reaction test results

Analyte name	Concentration (ng/ml)	Apparent FT3 level pg/ml
Iodothyrosine	10	<0.5
Phenylbutazone	10	<0.5
Sodium salicylate	10	<0.5
Diiodothyronine	10	<0.5
L-thyroxine	10	<0.5

#### 4. Test Recovery

To assess test recovery, certain amount of FT3 was added to 3 different sera with known concentration of FT3 and the sera were tested by Pishtaz teb FREE T3 ELISA test. The recovery was determined for each serum and results are shown below:

Table 4: Test recovery

No.	FREE T3 level pg/ml	FREE T3 added pg/ml	Exp. value pg/ml	Observed value pg/ml	Rec.%
1	1.8	1.5	1.65	1.84	111
1	1.8	3	2.4	2.6	108
1	1.8	6	3.9	4.06	104
2	3.4	1.5	2.45	2.35	95
2	3.4	3	3.2	3.35	104
2	3.4	6	4.7	4.95	105
3	7.5	1.5	4.5	4.2	93
3	7.5	3	5.25	5.0	95
3	7.5	6	6.75	6.9	102

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

#### References

1. Barjer, S.B., (1948) Determination of protein bound iodine. *J. Biol. Chem.* 173,175.
1. Chopra, I.j., Solomon, D.H. and Ho, R.S., (1971) A radioimmunoassay of triiodothyronine. *J. Clin. Endocrinol.* 33,865.
2. Young D.S., Prstaner, L.C. and Gilberman, U. (1975) Effects of drugs on clinical laboratory tests. *Clin. Chem.* 21, 3660.
3. Sterling, L. (1975) Diagnosis and treatment of thyroid disease, Cleveland CRC Press, 19-51.




#### FREE T3 Test Procedure

##### Step 1

Reagent	Standard	Control	Sample
Standards	50µl	--	--
Control serum	--	50µl	--
Sample	--	--	50µl
Assay buffer	50 µl	50 µl	50 µl

Shake wells gently for 15 seconds to mix content of the wells. Cover wells with cardboard sealer. Incubate for 30 minutes at room temperature.




##### Step 2

T3-HRP conjugate	50 µl	50 µl	50 µl
			

Shake wells gently for 15 seconds to mix content of the wells. Cover wells with cardboard sealer. Incubate for 30 minutes at room temperature.




##### Step 3

Remove plate cover and discard wells content. 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 the dark.

##### Step 4

Stop Solution	100 µl	100 µl	100 µl
			

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