

FREE T3 ELISA Kit

48 Tests Kit

Enzyme Immunoassay for the
Quantitative Determination of
FREE T3 Hormone Concentration
In Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-FT3-48

PISHTAZ TEB DIAGNOSTICS

Introduction

3,5,3' triiodothyronine (T3) hormone secreted by thyroid gland and circulate in the bloodstream, mostly (99.7%) bound to the plasma protein, thyroxine binding globulin (TBG), prealbumine (TBPA) and albumin. The remaining (0.3%) is free, 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 Free T3 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 T3 and conjugated T3 compete for the

limited binding sites on the wells. The wells are completely washed to remove unbound FREE T3.

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 FREE T3 in the sample. By reference to a series of FREE T3 standards assayed in the same way, the concentration of FREE T3 in the unknown sample is quantified.

Materials Provided with Test Kit

1. Antibody coated Wells (1 plate, 48 wells)
Microtiter wells coated with monoclonal anti Treeiodothyronine
2. Enzyme conjugates (1 vial, 3 ml):
Treeiodothyronine labeled with HRP in buffer contains protein as stabilizer and Thiomersal as preservative, ready to use.
3. Standard Set (0.5 ml / vial): Contain 0, 1.5, 3.0, 6, 12, and 20 pg/ml of FT3 in buffer contain protein as stabilizer and thiomersal as preservative, ready to use.
4. Control Serum (1 vial, 0.5 ml):
Contain certain amount of FT3 in buffer contain protein as stabilizer and thiomersal as Preservative, ready to use.
5. Assay buffer (1 vials, 3ml)
Contain Phosphate buffer solution with protein as stabilizer and Kathon CG as preservative, ready to use
6. Chromogen Substrate Reagent (1 vial, 6 ml):
Contain Tetra Methyl Benzedrine and hydrogen peroxide, ready to use.
7. Wash solution (1 vials, 25 ml concentrated 20x)
Contain Phosphate buffer solution with 0.05 % Tween 20
8. Stop solution (1 vial, 6 ml):
Contain 1 molar Hydrochloric acid
9. Cardboard sealer, 1 pieces

Materials/Equipments required but not provided with Test Kit

1. ELISA reader.
2. Precision pipettes: 50 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 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. 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 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 50µl of each standard, serum control and specimen into appropriate wells.
3. Add 50 µl 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 µl 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 µl 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 µl of chromogen-substrate (TMB) into the wells.
10. Leave the microplate for 15 minutes at room temperature and dark.
11. Add 100 µl 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)

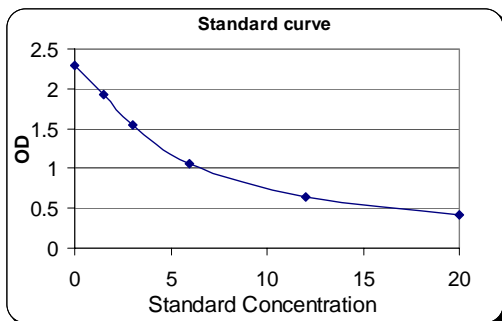
Results Calculation

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 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 T3 in pg/ml from the standard curve.

Standards Pg/ml	Absorbance
0	2.3
1.5	1.93
3	1.55
6	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 FREE T3 was determined by repeated ELISA tests on serum of normal people and results shown below, but it is highly recommended that each medical laboratory use their 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 FREE T3 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 four different serums with different FREE T3 concentration. Results are shown in table 1 and 2:

Table no. 1 (Intra-assay)

Specimen No.	Number of tests	Means pg/ml	SD pg/ml	CV%
1	24	1.25	0.05	4
2	24	2.72	0.13	4.7
3	24	7.75	0.31	4

Table No. 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, serums with different concentration of L-Thyroxine, Diiodothyronine, Diiodothyrosine, Iodothyrosine, Phenylbutazone and sodium Salicylate were tested. Results are shown in table 3.

Table no. 3 (Specificity test result, Cross reaction)

Analyte name	Concentration (ng/ml)	Apparent FREE T3 level
Iodothyrosine	10	<0.0001
Phenylbutazone	10	<0.0001
Sodium Salicylate	10	<0.0001
Diiodothyronine	10	<0.0001
L-thyroxine	10	<0.0001

4. Test Recovery

To assess test recovery, certain amount of FREE T3 was added into 4 different serums with known concentration of FREE T3 and the serums were tested by FREE T3 ELISA test. The recovery was determined for each serum and results are shown below:

Table no. 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

- Barjer, S.B., (1948) Determination of protein bound iodine. *J. Biol. Chem.* 173,175
- Chopra, I.j., Solomon, D.H. and Ho, R.S., (1971) A radioimmunoassay of triiodothyronine. *J. Clin. Endocrinol.* 33,865.
- Young D.S., Prstaner, L.C. and Gilberman, U. (1975) Effects of drugs on clinical laboratory tests. *Clin. Chem.* 21, 3660.
- 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	--	--
Serum Control	--	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.

TMB	100 µl	100 µl	100 µl
			

Incubate wells for 15 minutes at room temperature in 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.