

T3 ELISA Kit

96 Tests Kit

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

PISHTAZ TEB DIAGNOSTICS

Introduction

The human thyroid gland is a major component of the endocrine system. Thyroid hormones perform many important functions. Thyroxine (T4) and 3,5,3' triiodothyronine(T3) hormones circulate in the bloodstream, mostly bound to the plasma protein, thyroxine binding globulin (TBG). The concentration of T3 is much less than that of T4, but its metabolic potency is much greater.

T3 determinations are an important factor in the diagnosis of thyroid disease. T3 determination is also useful in monitoring both patients under treatment for hyperthyroidism and patients who have discontinued anti-thyroid drug therapy. It is especially valuable in distinguishing between euthyroid and hyperthyroid subjects. In addition to hyperthyroidism, T3 levels are elevated in women who are pregnant, and in women receiving oral contraceptives or estrogen treatment, paralleling TBG increases in a manner analogous to T4 levels. Likewise, a reduction in TBG concentration decreases T3 concentration. These changes in the T3 level, however, are not a true reflection of thyroid status.

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 assay buffer 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 T3. 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 T3 in the sample. By reference to a series of T3 standards assayed in the same way, the concentration of T3 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 Kathon CG as preservative, ready to use.
3. Standard Set (1 ml / vial): Contains 0, 0.5, 1.0, 2.5, 5, and 10 ng/ml of T3 in buffer containing protein as stabilizer and Kathon CG as preservative, ready to use.
4. Control serum (1 vial, 1 ml): Contains certain amount of T3 in buffer containing protein as stabilizer and Kathon CG as Preservative, ready to use.
5. Assay buffer (1 vial, 6ml): Contains Phosphate buffer solution with protein as stabilizer and Kathon CG as preservative, ready to use.
6. Chromogen Substrate Reagent (1 vial, 12 ml): Contains Tetra Methyl Benzidine and hydrogen peroxide, ready to use.
7. Wash solution (1 vial, 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.

10. Pipette tips.

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

Materials/Equipments required but not provided with Test Kit

1. ELISA reader.
2. Precision pipettes: 50 µl and 100 µl micropipettes.
3. Distilled water.
4. Vortex mixer or equivalent.
5. Absorbent paper.
6. 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 °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

Reagents Preparation

1. All reagents should be allowed to reach to 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 µl of each standard, control serum 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) in the dark.
6. Add 50 µl of HRP-T3 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 in the dark.
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 solution into the wells.
10. Leave the microplate for 15 minutes at room temperature and the 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

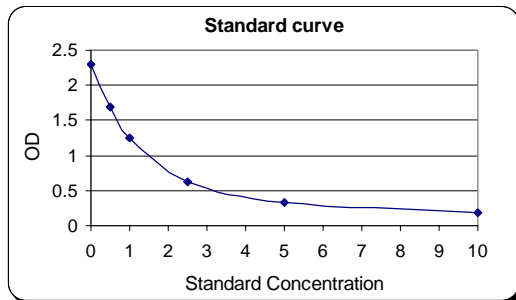
1. Measure absorbance of standards and



samples at 450 nm (and 630 nm as reference filter) and calculate mean of duplicate specimens.

- Construct a point to point standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/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 T3 in ng/ml from the standard curve.

Standards (ng/ml)	Absorbance
0	2.30
0.5	1.70
1	1.25
2.5	0.60
5	0.33
10	0.18



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 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 (ng/ml)	Mean (ng/ml)
0.6-2.1	1.4

$$\text{ng/ml} \times 100 = \text{ng/dL}$$

$$\text{ng/ml} \times 1.536 = \text{nmol/L}$$

$$\text{nmol/L} \times 0.651 = \text{ng/ml}$$

Performance Characteristics

1. Minimum Detection Limit

Based on mean absorbance of standard 0 - 3SD, the minimum concentration of T3 which is detected by kit is 0.1 ng/ml.

2. Test Precision

To determine intra-assay and inter-assay of kit, multiple tests were performed on three different sera with different T3 concentrations. Results are shown in table 1 and 2:

Table 1 (Intra-assay)

Specimen No.	Number of tests	Means ng/ml	SD ng/ml	CV %
1	24	0.53	0.025	4.7
2	24	1.63	0.05	3.1
3	24	5.45	0.17	3.1

Table 2 (Inter-assay)

Specimen No.	Number of tests	Means ng/ml	SD ng/ml	CV%
1	10	0.57	0.05	8.8
2	10	1.66	0.06	3.6
3	10	5.65	0.28	4.9

3. Test Specificity

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

Table 3 (Specificity test result, Cross reaction)

Analyte name	Concentration (µg/ml)	Apparent T3 level
Iodothyrosine	10	<0.1
Phenylbutazone	10	<0.1
Sodium Salicylate	10	<0.1
Diiodothyronine	10	<0.1
L-thyroxine	10	<0.1
Diiodothyrosine	10	<0.1

4. Test Recovery

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

Table 4: Test recovery

No.	T3 level ng/ml	T3 added ng/ml	Exp. value ng/ml	Observed value ng/ml	Rec.%
1	0.6	1	0.8	0.8	100
1	0.6	5	2.8	2.6	93
1	0.6	10	5.3	5.1	96
2	1.6	1	1.3	1.4	107
2	1.6	5	3.3	3.1	94
2	1.6	10	5.8	6.3	109
3	2.4	1	1.7	1.55	91
3	2.4	5	3.7	4.1	110
3	2.4	10	6.2	5.7	92
4	3.8	1	2.4	2.6	108
4	3.8	5	4.4	4.1	93
4	3.8	10	6.9	6.5	94

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

5. Test Linearity

To determine test linearity four different serum samples were diluted sequentially by zero standard. Then the sera were tested by this T3 ELISA kit. The results and serum recovery were determined considering dilution factor. The results are shown below:

Table 5: Test linearity

No.	T3 undiluted specimen	Recovery (%)			
		1:2	1:4	1:8	1:16
1	1.4	103	97	108	-
2	2.9	92	95	109	101
3	4.8	100	111	89	87
4	7.8	100	105	109	111

References

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

T3 Test Procedure
Step 1

Reagent	Standard	Control	Sample
Standards	50 µl	--	--
Control serum	--	50 µl	--
Sample	--	--	50 µl
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 in the dark.


Step 2

T3-HRP conjugate	50 µl	50 µl	50 µl
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Shake wells gently for 15 seconds to mix content of the wells. Cover wells with cardboard sealer. Incubate for 30 minutes at room temperature in the dark.

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
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Incubate wells for 15 minutes at room temperature in dark.


Step 4

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.