

T Uptake ELISA Kit

96 Tests Kit

Enzyme Immunoassay for the
Quantitative Determination of
Thyroid Uptake Test
Concentration in Human Serum/Plasma
(For In Vitro Diagnostic Use Only)
Catalogue No. PT-T-up-96

PISHTAZ TEB DIAGNOSTICS

Introduction

The thyroid gland under the regulatory control of thyrotropin hormone secretes thyroxine (T4) and triiodothyronine (T3) into the general circulation. The released hormones do not circulate as free molecules but are almost entirely (99.9%) bound to specific serum proteins.

Three protein fractions with varying affinities and capacities for interaction with T3 and T4 have been identified. Thyroxine binding globulin (TBG) carries 65~75% of the total circulating concentration. Thyroxine binding pre-albumin (TBPA) has an intermediate avidity for thyroxine (carries approx.15~25%) but little if any avidity for triiodothyronine. Albumin with a low affinity but high capacity carries 10% of thyroxine and 30% of the available triiodothyronine.

Since the metabolic processes are regulated entirely by the concentration of the free thyroid hormones, which are inversely related to the levels of the binding proteins, an assessment of the binding capacity of human serum was developed.

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations.

Test Principle

The test principle is based on competitive enzyme immunoassay. The microplate wells coated by certain amount of anti-T4 monoclonal antibody (mAb). Patient specimen or control is first added to a microplate wells. Enzyme-T4 conjugate and thyroxine (T4) are

then added and the reactants are mixed. Upon mixing the enzyme-conjugate and thyroxine with the specimen, a binding reaction results between the patient's binding proteins and the added thyroxine but not with the enzyme conjugate. The added thyroxine (T4) not consumed in reaction with patients binding proteins then competes with the enzyme-antigen conjugate for a limited number of immobilized coated anti-T4 binding sites. When equilibrium is attained, the antibody-bound fraction is separated from unbound enzyme-antigen by washing. The enzyme activity in the antibody-bound fraction is directly proportional to the binding capacity of the specimen. Thus, in hypothyroidism, the binding proteins are relatively unsaturated (due to the low level of thyroid hormones) resulting in higher consumption of the added thyroxine than a euthyroid specimen. This leads to higher binding of the enzyme-thyroxine conjugate caused by the reduced concentration of the available thyroxine. In hyperthyroidism, the reverse is true. The binding proteins are relatively saturated with thyroxine (due to the high level of thyroid hormone) resulting in lower consumption of the added thyroxine. The remaining thyroxine is relatively much higher than an euthyroid specimen resulting in lower enzyme-thyroxine antibody binding due to the increased competition of the thyroxine for the limited antibody sites.

The employment of several serum references of known unsaturated thyroid hormone binding capacity permits construction of a graph of absorbance and concentration. From comparison to the dose response curve, an unknown specimen's absorbance can be correlated with thyroid hormone binding.

Materials provided with the kit

1. Antibody coated Well (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 Thiomerosal as preservative, ready to use.
3. Standard Set (0.5 ml / vial): Contains 13, 23, 33, 43 approximate percentage of saturation of TBG in buffer containing protein as stabilizer and thiomerosal as preservative, ready to



- use. **Exact concentration of each standard is printed on standard's table.**
4. Assay buffer (1 vial, 6 ml): Contains phosphate buffer solution with protein as stabilizer and Kathon CG as preservative, ready to use.
 5. Chromogen substrate reagent (1 vial, 12 ml): Contains Tetra Methyl Benzedrine (TMB) and hydrogen peroxide, ready to use.
 6. Wash solution (1 vial, 50 ml concentrated 20x) Contains phosphate buffer solution with 0.05 % Tween 20.
 7. Stop solution (1 vial, 12 ml): Contains 1 molar Hydrochloric acid.
 8. Cardboard sealer.

2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use reagents beyond the kit expiration date.

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.

Materials required but not provided

1. Precision pipettes: 25 µl, 50µl and 100 µl
2. Disposable pipette tips.
3. Distilled water.
4. Absorbent paper or paper towel.
5. Graph paper.
6. Microplate reader with 450 nm and 630 nm wavelength absorbance capability.

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.

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.

Test Procedure

1. Secure the desired number of coated wells in the holder and keep the remaining with desiccants in tightly closed special bag.
2. Add 25 µl of each standard and sample in separate wells in duplicate format.
3. Add 50 µl of assay buffer to each well.
4. Dispense 50µl of T4-HRP conjugate to each well and mix reactants for 15 seconds. Cover the microplate wells with cardboard sealer and incubate them for 1 hour at room temperature (22-28°C).
5. Remove the sealer and take out the incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times (each time with 300 µl of working wash solution).

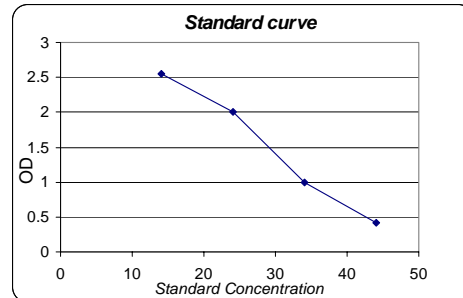
Storage Conditions

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.



6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Dispense 100 µl of chromogen/ substrate solution into the microplate wells.
8. Incubate the microplate wells at room temperature and dark for 15 minutes, to develop color.
9. Stop the reaction by adding 100 µl of stop solution to the microplate wells.
10. Measure absorbance at 450 nm by ELISA reader. (Use 630 nm filter as reference filter if it's available).

Fig.1: Example of T-Uptake standard curve



Note: All absorbances shown in above curve and table are for the purpose of illustration only, and should not be used to calculate unknowns. Users should obtain their own data and standard curves.

Result Calculation

1. Calculate mean absorbance value of standards and samples at 450 nm. (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 uptake in % on linear graph paper, with absorbance on the vertical (y) axis and binding capacity on the horizontal (x) axis.
3. Use the mean absorbance value for each sample; determine the corresponding of Uptake in % from the standard curve.

Expected Values

The normal value for T uptake was determined by repeated ELISA tests on sera of normal healthy population. But it is highly recommended that medical laboratories use their own normal references.

Table 2: T-Uptake Expected value

Reference interval %	Mean %
25-35	30

Example of Standard curve

Standards %	OD
15	2.55
26.5	2.01
35.5	0.99
46	0.42

Performance Characteristics

Test Precision

Three serum samples with different binding capacity were repeatedly tested by Pishtaz teb T-Uptake ELISA kit. Results are shown in table 3 and 4:

Table 3: Intra-assay

No.	No. of tests performed	Means %	SD %	CV %
1	24	22.0	0.70	3.2
2	24	25.4	0.64	2.5
3	24	35.3	0.79	2.2

Table 4: Inter-assay

No.	No. of tests performed	Means %	SD %	CV %
1	10	22.7	1.43	6.28
2	10	27.9	0.81	2.90
3	10	38.4	0.94	2.44




Each test has been run in duplicate

References

1. Inada, M. and Sterling K. (1967) *J. Clin. Invest.* 46,1442
2. Murphy, B., (1968) Radioisotopes in medicine, US Atomic Energy Commission, Technical information center, Tennessee.
3. Hollander, C.S. and Shenkman, L., (1974) Methods of hormone radioimmunoassay, Academic Press, New York.
4. Hamolsky, M.W., Stein, M. and Freedberg, S. A., (1957) *J. Clin. Endocrinol.*, 17, 33.
5. Herbert, V. (1971) U.S. Patent Office #3,442,819.
6. Mitchell, M. L., Harden, A. B. and O'Rourke, M.E. (1974) *J.Clin. Endocrinol.* 20, 1474.
7. Rolleri, E., Buzzigoli, G. and Plassio, C., (1982) *J. Nucl. Med.* 13, 892.
8. Nusynowitz, M.L. and Waliszewski, (1971) *Am. J. Clin. Pathol.* 56, 523.
9. Clark, F. and Horn, D.B., (1965) *J. Clin. Endocrinol. Metab.* 25, 39.
10. Young, D.S., Pestaner, L.C. and Giberman, U. (1975) *Clin. Chem.* 21, 3660.

T-Uptake Test Procedure

Step 1



Reagent	Standard	Sample
		
Standards	25 µl	None
Sample	None	25 µl
Assay Buffer	50 µl	50 µl
T4-HRP conjugate	50 µl	50 µl

Shake wells gently for 15 seconds to mix contents of the wells. Cover the microplate wells with cardboard sealer tightly and incubate them for 60 minutes at room temperature.



Step 2



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

Chromogen-substrate solution	100 µl 	100 µl 
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Incubate wells for 15 minutes at room temperature and dark.



Step 3

Stop Solution	100 µl 	100 µl 
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Read absorbance at 450 nm (Use 630 nm filter as reference filter if it's available).