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IVD See external label 2°C-8°C
Σ=96 tests REF **3176**

Thyroid Uptake Enzyme Immunoassay Kit

CATALOG NUMBER: 3176 (96 tests)

Intended Use: The Measurement of the Total Amount of Binding Sites Available for the Thyroid Hormones in Human Serum or Plasma by a Microplate Enzyme Immunoassay

SUMMARY AND EXPLANATION OF THE TEST

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 by reverse flow paper electrophoresis (1). 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 (1, 2, 3).

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 in 1957 by Hamolsky (4). In this early method, radioactive T3 was added to a specimen of whole blood. After an incubation period, the mixture was centrifuged and the red cells washed. The radioactivity uptake of the red cells was inversely related to the binding capacity of the serum. Although this method had severe limitations, it proved to be a valuable diagnostic tool.

Further technical improvements in the assay methodology of the T-uptake test resulted as various separation agents such as coated charcoal (5), ion-exchange resins (6), denatured albumin (7), silicates (8), antibodies and organic polymers were employed in place of the red cells.

This microplate enzyme immunoassay methodology provides the technician with optimum sensitivity while requiring few technical manipulations. In this method, serum reference, patient specimen, or control is first added to a microplate well. Enzyme-T4 conjugate and thyroxine (T4) are added, then the reactants are mixed. The endogenous binding proteins of the sample react with the thyroxine, **but not with the enzyme conjugate.** This leads to a higher binding of the enzyme conjugate to the antibody combining sites immobilized on the well as the binding capacity of the specimen increases.

After the completion of the required incubation period, the antibody bound enzyme-thyroxine conjugate is separated from the unbound enzyme-thyroxine conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

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 capacity

PRINCIPLE
Competitive Enzyme Immunoassay

The required components for assessing the binding capacity of human serum are enzyme-thyroxine conjugate, thyroxine, binding protein (P), and immobilized thyroxine antibody (Ab).

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**. This interaction is represented below:

$$T4 + P \rightleftharpoons T4\text{-}P \quad (1)$$

T4 = Thyroxine added (constant quantity)
P = Specific binding proteins (varying quantity)

The added thyroxine (T4) not consumed in reaction 1 then competes with the enzyme-antigen conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}T4 + T4_r + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} T4Ab_{C.W.} + {}^{Enz}T4Ab_{C.W.} \quad (2)$$

$Ab_{C.W.}$ = Monospecific Immobilized T4 Antibody (Constant Quantity)
$T4_r$ = Added thyroxine unreacted in reaction (1) (Variable Quantity)
$^{Enz}T4$ = Enzyme-antigen Conjugate (Constant Quantity)
$T4Ab_{C.W.}$ = Thyroxine-Antibody Complex
$^{Enz}T4\ Ab_{C.W.}$ = Enzyme-thyroxine Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound enzyme-antigen by decantation or aspiration. 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.

REAGENTS FOR 96-well MICROPLATE

A. Human Serum References -- 1.0 ml/vial - Icons A-D
Four (4) vials of serum reference of unsaturated thyroid hormone binding capacity at approximate* levels of 15.0 (**A**), 26.5 (**B**), 35.5 (**C**), and 46.0 (**D**) %U. Store at 2-8°C. A preservative has been added.
* Exact levels are given on the labels on a lot specific basis.

B. Enzyme-T4 Conjugate – 1.0ml/vial - Icon E
One (1) vial of thyroxine-horseradish peroxidase (HRP) conjugate and thyroxine in a bovine albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C. T-Uptake Conjugate Buffer-- 10 ml - Icon B
One (1) bottle reagent containing buffer, orange dye and preservative. Store at 2-8°C.

D. Antibody Coated Microplate -- 96 wells - Icon
One 96-well microplate coated with sheep anti-thyroxine serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate -- 20ml - Icon
One (1) vial containing a surfactant in phosphate buffered saline. A preservative has been added. Store at 2-30°C.

F. Substrate A --7.0ml/vial - Icon S^A
One (1) bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B -- 7.0ml/vial - Icon S^B
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution -- 6.0ml/vial - Icon 

One (1) bottle containing a strong acid (1N HCl). Store at 2-30°C.

Note 1: Do not use reagents beyond the kit expiration date.

Note 2: Opened reagents are stable for sixty (60) days when stored at 2-8°C.

PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

SPECIMEN COLLECTION AND PREPARATION

Collect sample(s) by venipuncture in ten (10) ml silicone evacuated tube(s) or evacuated tube(s) containing EDTA or heparin. The usual precautions in the collection of venipuncture samples should be observed. Separate the red blood cells by centrifugation use serum or plasma for the T-Uptake procedure. Specimen(s) may be refrigerated at 2-8°C for a maximum period of 48 hours. If the specimen(s) can not be assayed within 48 hours, the sample(s) may be stored at temperatures of -20°C for up to 30 days. When assayed in duplicate, 0.05ml of the specimen is required.

The cross-reactivity of the thyroxine antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of thyroxine needed to displace the same amount of tracer.

Substance		Cross Reactivity	Concentration
l -Thyroxine		1.0000	---
d-Thyroxine		0.9800	10µg/dl
d-Triiodothyronine	0.0150	100µg/dl	
l -Triiodothyronine	0.0300	100µg/dl	
lodothyrosine		0.0001	100µg/ml
Diiodothyrosine	0.0001	100µg/ml	
Diiodothyronine	0.0001	100µg/ml	

MATERIALS
Provided:

1. Four (4) vials of T-uptake human serum references.
2. One (1) vial of Thyroxine-enzyme Conjugate Concentrate.
3. One (1) vial of T-Uptake Conjugate Buffer.
4. One 96-well Antibody Coated Microplate.
5. One (1) bottle of Wash buffer concentrate.
6. One (1) bottle of Substrate A.
7. One (1) bottle of Substrate B.
8. One (1) bottle of Stop solution.
9. Instructions.

Required But Not Provided:

1. Pipette capable of delivering 25µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.300ml volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200µl) and (200-1000µl) dispenser(s) for conjugate and substrate dilutions
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Test tubes for dilution of enzyme conjugate and substrate A and B.
7. Absorbent Paper for blotting the microplate wells.
8. Plastic wrap or microplate cover for incubation steps.
9. Vacuum aspirator (optional) for wash steps.
10. Timer.
11. Quality control materials.

REAGENT PREPARATION:

1. Working Reagent 1 – T4-enzyme Solution – Prepare Monthly
Dilute the Thyroxine-enzyme conjugate 1:11 with T-Uptake enzyme conjugate buffer in a suitable container. For example, dilute 160µl of conjugate with 1.6ml of buffer for 16 wells (A slight excess of solution is made). This reagent should be used within thirty (30) days for maximum performance of the assay. Store at 2-8°C.
General Formula:
Amount of Buffer required = Number of wells * 0.1
Quantity of Enzyme conjugate necessary = # of wells * 0.01
i.e. = 16 x 0.1 = 1.6ml for T4 Conjugate Buffer
16 x 0.01 = 0.16ml (160µl) for T4 enzyme conjugate

2. Wash Buffer
Dilute contents of Wash Concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature until expiration date printed on concentrate label. **It is essential that all the contents of the wash buffer concentrate dissolve. Crystal formation in the Wash Concentrate can be eliminated by briefly (approx. 5 minutes) heating in a water bath at 37°C or storing the Wash Concentrate at room temperature**.

3. Working Substrate Solution – Prepare daily
Determine the amount of reagent needed and prepare by mixing equal portions of Substrate A and Substrate B in a suitable container. For example, add 1ml of A and 1ml of B per two (2) eight well strips (A slight excess of solution is made). **Use within twenty-four hours of preparation for maximum performance of the assay.**

TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20 - 27°C).

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate.

   Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
2. Pipette 0.025 ml (25µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Working Reagent 1, T4-enzyme solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 300µl of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

QUALITY CONTROL

Each laboratory should assay controls at levels in the hypothyroid, euthyroid and hyperthyroid range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

Q.C. PARAMETERS

Maximum Absorbance (O calibrator) = > 1.2

RESULTS

A dose response curve is used to ascertain the unsaturated thyroid binding capacity in unknown specimens.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding %T-Uptake (%U) on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with the best-fit curve.
4. To determine the %T-uptake for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the reference response, and read the %T-uptake (%U) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 1.698 (intersects the reference curve at (27.3%U) (See Figure 1).

EXAMPLE 1

WELL	SERUM REFERENCES	ABSORBANCE
1	15.0 %U	2.605
2	15.0 %U	2.638
3	26.5 %U	1.802
4	26.5 %U	1.719
5	35.5 %U	1.082
6	35.5 %U	1.030
7	46.0 %U	0.582
8	46.0 %U	0.547

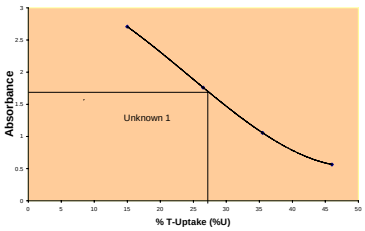
Well	Unknown I. D.	O.D.	Avg. O.D.	Value
9	Unknown #1	1.701		
10	Unknown #1	1.695	1.698	27.3%U

The T-Uptake can also be expressed as a T-uptake Ratio. Divide the %U by 30% to convert into a T-Uptake ratio.

EXAMPLE 2

27.3%U / 30% = 0.910

Figure 1



*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a standard curve prepared with each assay

PERFORMANCE CHARACTERISTICS

A. Precision

The within and between assay precision of the T-Uptake Microplate EIA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2

Within Assay Precision (Values in %U)

Sample	N	X	σ	C.V.
Low	24	28.7	0.393	1.37%
Normal	24	37.8	0.515	1.36%
High	24	45.4	0.332	0.73%

TABLE 3

Between Assay Precision (Values in %U)

Sample	N	X	σ	C.V.
Low	10	28.4	0.45	1.6%
Normal	10	37.1	0.65	1.8%
High	10	45.7	0.52	1.1%

*As measured in ten experiments in duplicate over a ten day period.

B. Accuracy (Method Comparison)

The T-Uptake Microplate EIA Test System was compared with a T3 Uptake radioassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid and pregnancy populations were used (The values ranged from 14% – 48%U). The total number of such specimens was 120. The least square regression equation and the correlation coefficient were computed for this T-Uptake EIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	29.3	y = 1.56+0.956(x)	0.972
Reference	29.0		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

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