



MP Biomedicals
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T3 Uptake ChLIA Kit

Catalog No.: 07M575A, 07M575B

INTENDED USE

The MP Biomedicals T3 Uptake ChLIA Kit is intended to be used for the measurement of the total amount of binding sites available for the Thyroid Hormones in Human Serum or Plasma by a Microplate Chemiluminescence Immunoassay (ChLIA). This test is for in vitro diagnostic use only.

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 T3-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 chemiluminescence 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-T3 conjugate and thyroxine (T4) are added, and 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, reactive for triiodothyronine and thyroxine, immobilized on the well as the binding capacity of the specimen increases.

After the completion of the required incubation period, the antibody bound enzyme conjugate is separated from the unbound enzyme 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 light.

The employment of several serum references of known unsaturated thyroid hormone binding capacity permits construction of a graph of light 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 (Type 5)

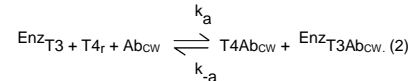
The required components for assessing the binding capacity of human serum are enzyme-T3 conjugate, thyroxine, binding protein (P), and immobilized thyroxine antibody (Ab). Upon mixing the enzyme-conjugate and thyroxine with the specimen, a binding reaction results between the patient's binding proteins and the added thyroxine but not with the enzyme conjugate. This interaction is represented by



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-T3 conjugate for a limited number of insolubilized binding sites. The interaction is illustrated by the following equation:



Ab_{CW} = Immobilized Antibody (Constant Quantity)

T₄ = Added T4 unreacted in reaction (1) (Variable Quantity)

Enz-T₃ = Enzyme-antigen Conjugate (Constant Quantity)

T₄-Ab_{CW} = Thyroxine-Antibody Complex

Enz-T₃-Ab_{CW} = Enzyme-T3 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, determined by reaction with a substrate that generates light, 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-triiodothyronine 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-antigen antibody binding due to the increased competition of the thyroxine for the limited antibody sites.

REAGENTS

Materials Provided:

A. Human Serum References – 1 mL/vial

Four (4) vials of serum reference of unsaturated thyroid hormone binding capacity at approximate* levels of 18 (A), 25 (B), 35 (C), and 47 (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-T3U Tracer – 1.5 mL/vial

One (1) vial of triiodothyronine-horseradish peroxidase (HRP) conjugate and thyroxine in an albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.

C. T3-Uptake Tracer Buffer – 13 mL

One (1) bottle reagent containing buffer, orange dye and preservative. Store at 2-8°C.

D. Light Reaction Wells – 96 wells

One 96-well white 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 – 20 mL

One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Signal Reagent A – 7 mL/vial

One (1) bottle containing luminol and an enhancer in buffer. Store at 2-8°C.

G. Signal Reagent B – 7 mL/vial

One (1) bottle containing hydrogen peroxide (H₂O₂) in buffer. Store at 2-8°C.

H. Product Insert

Required But Not Provided:

1. Pipette capable of delivering 25 µL volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 mL and 0.350 mL volumes with a precision of better than 1.5%.
3. Adjustable volume (20-200 µL) and (200-1000 µL) dispenser(s) for conjugate and substrate dilutions.
4. Microplate Luminometer.
5. Microplate washers or a squeeze bottle (optional).
6. Test tubes for dilution of enzyme conjugate and Signal Reagent 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.

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

Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**

Note 3: Above reagents are for a single 96-well microplate.

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.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or plasma in type, and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050 mL of the specimen is required.

REAGENT PREPARATION

1. Working T3U-Enzyme Tracer Solution

Dilute the T3U-enzyme Tracer 1:11 with T3 Uptake Tracer 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 twenty-four hours for maximum performance of the assay. Store at 2-8°C.

General Formula:

$$\begin{aligned} \text{Amount of Buffer required} &= \text{Number of wells} \times 0.1 \\ \text{Quantity of T3-Enzyme necessary} &= \# \text{ of wells} \times 0.01 \\ \text{i.e.} &= 16 \times 0.1 = 1.6 \text{ mL for T3 Conjugate Buffer} \\ &= 16 \times 0.01 = 0.16 \text{ mL (160 } \mu\text{L) for T3 enzyme conjugate} \end{aligned}$$

2. Wash Buffer

Dilute contents of Wash Concentrate to 1000 mL with distilled or deionized water in a suitable storage container. Store diluted buffer at 2-30°C for up to 60 days.

3. Working Signal Reagent Solution - Store at 2 - 8°C.

Determine the amount of reagent needed and prepare by mixing equal portions of Signal Reagent A and Signal Reagent B in a clean container. For example, add 1 mL of A and 1 mL of B per two (2) eight well strips (A slight excess of solution is made). **Discard the unused portion if not used within 36 hours after mixing.** If complete utilization of the reagents is anticipated, within the above time constraint, pour the contents of Signal Reagent B into Signal Reagent A and label accordingly.

Note: Do not use reagents that are contaminated or have bacteria growth.

TEST PROCEDURE

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

****Test Procedure should be performed by a skilled individual or trained professional****

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 (25 µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 mL (100 µL) of Working Reagent 1, T3U-enzyme tracer solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 45 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 350 µL of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat four (4) additional times for a total of five (5) 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 four (4) additional times.**
8. Add 0.100 mL (100 µL) of working Signal Reagent to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate for five (5) minutes in the dark.
10. Read the Relative Light Units (RLU's) in each well using a microplate luminometer. **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 low, normal and elevated ranges for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control records should be maintained and used to monitor batch to batch consistency.

CALCULATION OF RESULTS

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

- Record the RLU's obtained from the printout of the microplate reader as outlined in Example 1.
- Plot the RLU's for each duplicate serum reference versus the corresponding %T3-Uptake (%U) on linear graph paper (do not average the duplicates of the serum references before plotting).
- Connect the points with the best-fit curve.
- To determine the %T3-uptake for an unknown, locate the average RLU's 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 RLU 42201 (intersects the reference curve at 30.8%U) (See Figure 1).

Note: Computer data reduction software designed for chemiluminescence assays may also be used for the data reduction. **If such software is utilized, the validation of the software should be ascertained.**

EXAMPLE 1

Sample ID.	Well Number	RLU (A)	Mean RLU (B)	Value (%U)
Cal A	A1	99062	100000	18.0%
	B1	100938		
Cal B	C1	73568	73173	25.5%
	D1	72777		
Cal C	E1	28006	28174	34.0%
	F1	28341		
Cal D	G1	7537	7697	47.5%
	H1	7857		
Pat 1	A2	42608	42201	30.8%
	B2	41793		

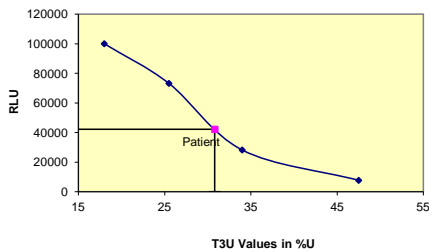
The T-Uptake can also be expressed as a T-uptake Ratio. Divide the %U by 30% to convert into a T-Uptake ratio.

EXAMPLE 2

$$27.3\%U / 30\% = 0.910$$

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay. In addition, the RLU's of the calibrators have been normalized to 100,000 RLU's for the A calibrator (greatest light output). This conversion minimizes differences caused by efficiency of the various instruments that can be used to measure light output.

Figure 1



LIMITATIONS OF PROCEDURE

Assay Performance

- It is important that the time of reaction in each well is held constant to achieve reproducible results.
- Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
- Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
- If more than one (1) plate is used, it is recommended to repeat the dose response curve.
- The addition of signal reagent initiates a kinetic reaction, therefore the signal reagent(s) should be added in the same sequence to eliminate any time-deviation during reaction.
- Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
- Use components from the same lot. No intermixing of reagents from different batches.
- Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from MP Biomedicals' IFU may yield inaccurate results.
- All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
- It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.

Interpretation

- Measurements and interpretation of results must be performed by a skilled individual or trained professional.**
- Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
- For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
- If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, **MP Biomedicals shall have no liability.**
- If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
- The T3-Uptake Test is dependent upon a multiplicity of factors: thyroid gland and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of the thyroid hormones to TBG. Thus, the T3-Uptake Test alone is not sufficient to assess clinical status.
- The free thyroxine index (FTI), which is the product of the T-Uptake Ratio and the total thyroxine concentration, has gained wide clinical acceptance as a more accurate assessment of thyroid status (9). The FTI value compensates for any condition or drug, such as pregnancy or estrogens, which alters the TBG and the T4 levels but does not change the thyrometabolic status. A table of interfering drugs and conditions, which affect the T3-Uptake Test, has been compiled by the Journal of the American Association of Clinical Chemists (10).

EXPECTED RANGES OF VALUES

A study of an euthyroid adult population (85 specimens) was undertaken to determine expected values for the T3 Uptake and are presented in Table 1.

TABLE 1
Expected Values for the T3 Uptake ChLIA

Thyroid Status	% T-	
	Uptake	T-Ratio
Euthyroid	25 - 35	0.83-1.17
Hypothyroid or TBG excess binding	< 25	< 0.83
Hyperthyroid or TBG saturation	>35	> 1.17

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and

the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

PERFORMANCE CHARACTERISTICS

Precision

The within and between assay precision of the T3 Uptake ChLIA test 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	25.8	0.26	1.1%
Normal	24	35.8	0.87	2.4%
High	24	45.1	0.38	0.9%

TABLE 3
Between Assay Precision (Values in %U)

Sample	N	X	σ	C.V.
Low	10	26.1	0.28	1.1%
Normal	10	35.5	1.22	3.4%
High	10	45.6	0.70	1.5%

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

Accuracy (Method Comparison)

The T3 Uptake ChLIA test was compared with a T3 Uptake enzyme immunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid and pregnancy populations were used (The values ranged from 15% - 45%U). The total number of such specimens was 105. The least square regression equation and the correlation coefficient were computed for this T3 Uptake ChLIA test 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	30.2	$y = 1.06 + 0.978(x)$	0.987
Reference	29.8		

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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