Monobind Inc.®
Lake Forest, CA 92630, USA

AccuLite®
CLIA Microwells

Triiodothyronine (T3)
AccuLite® CLIA Test System
Product Code: 175-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Triiodothyronine Concentration in Human Serum or Plasma by a Microplate Chemiluminescence Immunoassay (CLIA)

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of serum triiodothyronine concentration is generally regarded as a valuable tool in the diagnosis of thyroid dysfunction. This importance has provided the impetus for the significant improvement in assay methodology that has occurred in the last two decades. The advent of monospecific antiserum and the discovery of blocking agents to the T3 binding serum proteins have enabled the development of procedurally simple radioimmunoassay (1,2).

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-T3 conjugate is added, and then the reactants are mixed. A competition reaction results between the enzyme conjugate and the native triiodothyronine for a limited number of antibody combining sites immobilized on the well.

After the completion of the required incubation period, the antibody bound T3-enzyme conjugate is separated from the unbound T3-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 triiodothyronine concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with T3 concentration.

3.0 PRINCIPLE

Competitive Chemiluminescence Immunoassay (Type 5)
The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubilized binding sites. The interaction is illustrated by the followed equation:

$$Enz_{Ag} + Ag + Ab_{C.W.} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{C.W.} + Enz_{AgAb_{C.W.}}$$

$Ab_{C.W.}$= Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
Enz_{Ag} = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{C.W.}$ = Antigen-Antibody Complex
$Enz_{Ag\,Ab_{C.W.}}$ = Enzyme-antigen 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 antigen by decantation or aspiration. The enzyme activity, determined by reaction with a substrate that generates light, in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. T3 Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for triiodothyronine at concentrations of 0 (**A**), 0.5 (**B**), 1.0 (**C**), 2.5 (**D**), 5.0(**E**) and 7.5(**F**) ng/ml. Store at 2-8°C. A preservative has been added.

B. T3 Tracer Reagent – 1.5ml/vial - Icon Ⓔ
One (1) vial of T3-horseradish peroxidase (HRP) conjugates in an albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C

C. T3/T4 Tracer Buffer – 13ml - Icon Ⓑ
One (1) bottle reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.

D. T3 Light Reaction Wells – 96 wells - Icon
One 96-well white microplate coated with sheep anti-T3 serum and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Concentrate – 20ml - Icon
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 - Icon C[A]
One (1) bottle containing Luminol in buffer. Store at 2-8°C.

G. Signal Reagent B – 7 ml/vial - Icon C[B]
One (1) bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Product Insert.

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

4.1 Materials [Required But Not Provided]:
1 Pipette capable of delivering 50µl volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml and 0.350ml 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 washers or a squeeze bottle (optional).
5. Microplate Luminometer.
6. Test tubes for dilution of enzyme conjugate and signal 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.

5.0 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.

6.0 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 (for serum) or evacuated tube(s) containing EDTA or heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma 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, 0.100ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay external 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 light intensity 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.

8.0 REAGENT PREPARATION

1. **Working Tracer - T3-enzyme Conjugate Solution**
Dilute the T3-Tracer 1:11 with Total T3/T4 Tracer buffer in a clean 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:
Amount of Buffer required = Number of wells * 0.1
Quantity of T3-Enzyme necessary = # of wells * 0.01
i.e. = 16 x 0.1 = 1.6ml for Total T3/T4 Conjugate Buffer
16 x 0.01 = 0.16ml (160µl) for T3 enzyme conjugate
2. **Wash Buffer**
Dilute contents of Wash Concentrate to 1000ml 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 1ml 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.

9.0 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 0.050 ml (50µl) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of Working Tracer, T3-enzyme conjugate solution to all wells (see Reagent Preparation Section).
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 350µl 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 (100µl) of working signal reagent solution 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 SIGNAL ADDITION
9. Incubate for five (5) minutes in the dark at room temperature
10. Read the relative light units in each well, for minimum 0.5 – 1.0 seconds, using a microplate luminometer. **The results should be read within thirty (30) minutes of adding the signal solution.**

Note: For re-assaying specimens with concentrations greater than 7.5 ng/ml, pipette 25µl of the specimen and 25µl of the 0 serum reference into the sample well (this maintains a uniform protein concentration). Multiply the readout value by 2 to obtain the triiodothyronine concentration.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of triiodothyronine in unknown specimens.
1. Record the RLU's obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the RLU's for each duplicate serum reference versus the corresponding T3 concentration in ng/ml on linear graph paper.
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of T3 for an unknown, locate the average RLU's for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average RLU's (63817) of the unknown intersects the calibration curve at (1.4 ng/ml) T3 concentration (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. Duplicates of the unknown may be averaged as indicated (See Figure 1).

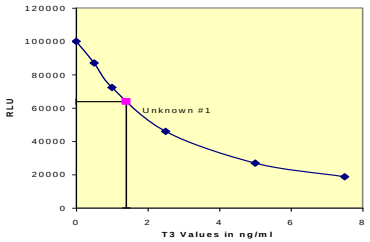
EXAMPLE 1

Sample I.D.	Well Number	RLU /s(A)	Mean RLU/s (B)	Value (ng/ml)
Cal A	A1	100468	100000	0
	B1	99532		
Cal B	C1	88644	86989	0.5
	D1	85333		
Cal C	E1	71996	72275	1.0
	F1	72553		
Cal D	G1	46658	45952	2.5
	H1	45247		
Cal E	A2	26556	26887	5.0
	B2	27219		

Cal F	C2	19549	18710	7.5
	D2	17871		
Ctrl 1	E2	71096	71745	***1.0***
	F2	72393		
Ctrl 2	G2	49482	49732	***2.2***
	H2	49981		
Patient	A3	64506	63817	***1.4***
	B3	63127		

*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/sec 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



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The Dose Response Curve should be within established parameters.
2. If used, 2 of 3 quality control pools should be within the established range.

12.0 RISK ANALYSIS

The SDS is available at https://www.monobind.com/safety-data-sheets and the Risk Analysis Form may be requested.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. 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.
6. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
7. Use components from the same lot. No intermixing of reagents from different batches.
8. Patient specimens with T3 concentrations above 7.5 ng/mL may be diluted ½ with '0' serum reference. The sample's concentration is obtained by multiplying the result by the dilution factor, 2.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. 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.
11. 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.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. 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.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. 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, Monobind shall have no liability.
5. 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.
6. Total serum triiodothyronine concentration is dependent upon a multiplicity of factors: thyroid gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of triiodothyronine to TBG (3, 4). Thus, **total triiodothyronine concentration alone is not sufficient to assess clinical status**.
7. A decrease in total triiodothyronine values is found with protein-wasting diseases, certain liver diseases and administration of testosterone, diphenylhydantoin or salicylates. A table of interfering drugs and conditions, which affect total triiodothyronine values, has been compiled by the Journal of the American Association of Clinical Chemists[3].

13.0 EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for T3 AccuLite® CLIA method. The mean (R) values standard deviations (σ) and expected ranges (±2σ) are presented in Table 1. The total number of samples was 85.

TABLE I
Expected Values for the T3 AccuLite® CLIA (in ng/ml)

Mean (X)	**1.22**
Standard Deviation (σ)	0.35
Expected Ranges (±2σ)	0.52 – 1.98

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.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the T3 AccuLite® CLIA assay were determined by analyses on three different levels of pool control sera. The number, mean value, standard deviation (σ) 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 ng/ml)

Sample	N	X	σ	C.V.
Low	20	0.80	0.05	6.3%
Normal	20	1.56	0.09	5.8%
High	20	3.06	0.10	3.3 %

TABLE 3
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	20	0.81	0.08	9.9%
Normal	20	1.57	0.16	10.2%
High	20	2.89	0.27	9.3%

*As measured in several experiments in duplicate.

14.2 Sensitivity

The Triiodothyronine test system procedure has a sensitivity of 0.126 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The T3 AccuLite® CLIA test was compared with a reference enzyme immunoassay method. Biological specimens from hypothyroid, euthyroid and hyperthyroid populations were used (The values ranged from 0.010ng/ml – 7.30ng/ml). The total number of such specimens was 110. The least square regression equation and the correlation coefficient were computed for the T3 AccuLite® CLIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Lease Square Regression Analysis	Correlation Coefficient
This Method	1.35	y = -15.0 + 1.085(x)	0.972
Reference	1.38		

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.

14.4 Specificity

The cross-reactivity of the triiodothyronine 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 triiodothyronine needed to displace the same amount of tracer.

Substance	Cross Reactivity	Concentration
I-Triiodothyronine	1.0000	-
I-Thyroxine	< 0.0002	10µg/ml
Iodothyrosine	< 0.0001	10µg/ml
Diiodothyrosine	< 0.0001	10µg/ml
Diiodothyronine	< 0.0001	10µg/ml
Phenylbutazone	< 0.0001	10µg/ml
Sodium Salicylate	< 0.0001	10µg/ml

15.0 REFERENCES

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2. Chopra IJ, Ho RS & Lam R, "An improved radio-immunoassay of triiodothyronine in human serum", *J Lab Clinical Med,* **80**, 729 **(**1971).
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4. Sterling L, Diagnosis and Treatment of Thyroid Disease, Cleveland, CRC Press, **9, 51** (1975).
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9. Bartalena L, "Recent achievements in studies on thyroid hormone-binding proteins*", Endocrine Rev,* **11,** 47-64(1990).
10. Engler D, Burger AG, "The deiodination of the iodothyronines and of their derivatives in man*", Endocrine Rev*, **5,** 151 (1984).

16.0 AVAILABLE CONFIGURATIONS

Available test-system sizes and included materials are shown below. Each "pack size" has a unique item/SKU number, which includes the product code. For the standard pack size of 96-microwells, the product code will have the letter "A" added (e.g., 175-300A) to make a SKU for sale. This test kit is currently available as per below (with contents shown in table):

ITEM #	DESCRIPTION
175-300A	T3 AccuLite CLIA Kit - 96 wells
175-300B	T3 AccuLite CLIA Kit - 192 wells
175-300D	T3 AccuLite CLIA Kit - 480 wells
175-300E	T3 AccuLite CLIA Kit - 960 wells

Size		96(A)	192(B)	480(D)	960(E)
Reagent (fill)	A)	1ml set	1ml set	2ml set	2ml set x2
	B)	1 (1.5ml)	2 (1.5ml)	1 (8ml)	2 (8ml)
	C)	1 (13ml)	2 (13ml)	1(60ml)	2 (60ml)
	D)	1 plate	2 plates	5 plates	10 plates
	E)	1 (20ml)	1 (20ml)	1 (60ml)	2 (60ml)
	F)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)
	G)	1 (7ml)	2 (7ml)	1 (30ml)	2 (30ml)

Also Available: QSure® Multi-Ligand Control

Revision: 5 **Date: 2025-NOV-14** **DCO: 1747**
MP175.5 **Product Code: *175-300***

For Orders and Inquires, please contact

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IVD 2° 8° CE

Please visit our website to learn more about our products and services.

Glossary of Symbols
(ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2° 8° — Temperature Limitation Storage Condition (2-8°C)
Used By (Expiration Day)
Consult Instructions for Use
REF — Catalogue Number
LOT — Batch Code
EU REP — Authorized Rep in European Country
Date of Manufacturer
Manufacturer
Do Not Use if Package is Damaged
Keep Away from Sunlight
Σ — Contains Sufficient Test for Σ
CE — European Conformity