BeneSphera™

T3 Microwell CLIA (IMF)

INTENDED USE

The **BeneSphera™ T3** (IMF) CLIA test is designed for the quantitative determination of total triiodothyronine concentration in human serum.

INTRODUCTION

The human thyroid gland is a major component of the endocrine system. Thyroid hormones perform many important functions, They exert powerful and essential regulatory influences on growth, differentiation, cellular metabolism and general hormonal balance of the body, as well as on the maintenance of metabolic activities and the development of the skeletal and organ system.

The hormones thyroxine (T4) and 3, 5, 3, triiodothyronine (T3) 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 determination is an important factor in the diagnosis of thyroid diseases. Its measurement has uncovered a' variant of hyperthyroidism in thyrotoxic patients with elevated T3 levels and normal T4 levels. An increase in T3 without an increase in T4 is frequently a forerunner of recurrent thyrotoxicosis in previously treated patients. The clinical significance of T3 is also evidentin patients in whom euthyroidism is attributable only to normal T3 although their T4 values are subnormal.

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.

PRINCIPLE OF THE TEST

In the T3 assay, a certain amount of T3 analog is coated on microtiter wells. A measured amount of patient serum, and a constant amount of anti-T3 antibody conjugated with horseradish peroxidase are added to the microtiter wells, 8-anilino-l-napthalene sulfonate (ANS) is used to displace T3 from proteins to enable the measurement of total circulating T3. During the incubation T3 analog on microtiter wells and T3 present in the samples and standards compete for binding to the anti-T3 monoclonal antibody horseradish peroxidase conjugate. After a 60 minutes incubation at 37°C, the wells are washed by Wash Solution. Upon the addition of the substrate, the horseradish peroxidase activity bound on the wells is then assayed by chemiluminescence reaction. The Related Light Unit (RLU) is inversely related to the concentration of T3 in the test sample.

KIT CONTENTS

1. **Coated Wells-** 1
   Microplate with T3 analog coated wells.
2. **Calibrators-** 2A - 2F
   Six bottles of human serum reference containing the following concentration of T3 with preservatives.
   (A) Calibrator 0.0 ng/ml (B) Calibrator 0.5 ng/ml
   (C) Calibrator 1.0 ng/ml (D) Calibrator 2.5 ng/ml
   (E) Calibrator 5.0 ng/ml (F) Calibrator 10.0 ng/ml
3. **Enzyme Conjugate Concentrate:** 3A
   Horseradish Peroxidase (HRP) labeled anti-T3 in Stabilizing Buffer.
4. **Enzyme Conjugate Diluent** 3B
5. **Wash Buffer Concentrate / PBS-T Powder:** 4
   PBS-Tween
6. **Substrate A** 5A
   The reagent contains LuminGlo chemiluminescent substrate in 2.0% Dimethyl sulfoxide.
7. **Substrate B** 5B
   The reagent contains LuminGlo chemiluminescent substrate buffer with 30% hydrogen peroxide.
8. **Insert - 01 No.**
9. **Microwell Plate Cover**
10. **Zip Seal Polybag**

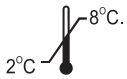
MATERIALS REQUIRED (BUT NOT PROVIDED)

1. Pipette capable of delivering 10 µl to 100 µl and 100 µl to 1000 µl
2. Disposable pipette tips
3. Microplate washer
4. Microplate luminometer
5. Absorbent paper for blotting the microplate wells
6. Timer
7. Quality control materials
8. Deionised water or purified water
9. Clean containers for mixing of reagents
10. Disposable gloves

PRECAUTIONS ⚠

- Reagents are for *in vitro* diagnostic use only IVD. Do not use after expiration date.
- Do not mix reagents from other kits with different lot numbers.
- Avoid cross contamination between reagents to ensure valid test results.
- Follow the wash procedure to ensure optimum assay performance.
- Use Plate Sealer to cover microwell plate during incubation to minimize evaporation.
- Use a new pipette tip for each specimen assayed.
- Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Do not allow wells to dry out during the assay procedure.
- Do not touch the bottom of the wells with pipette tips.
- Do not allow sodium hypochlorite fumes from chlorine bleach or other sources to contact the microwell plate during the assay as the substrate reaction may be inhibited.
- All equipment should be used with care, calibrated regularly and maintained following the equipment manufacturer's instructions.
- Some components of this kit contain human blood derivatives. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents.

- Therefore, all blood derivatives should be considered potentially infectious. It is recommended that these reagents and human specimens be handled using established good laboratory working practices.
- Wear disposable gloves and other protective clothing such as laboratory coats and eye protection while handling kit reagents and specimens. Wash hands thoroughly when finished.
- Proclin 300 is included as a preservative in the Enzyme conjugate, Wash Buffer, Substrate and Calibrators. Avoid any contact with skin or eyes.
- Do not eat, drink or smoke in the area where the specimens or kits are handled. Do not pipette by mouth.
- Handle and dispose all specimens and materials used to perform the test as if they contained infectious agents. Observe established precautions against microbiological hazards throughout all the procedures and follow the standard procedures for proper disposal of specimens.



STORAGE AND STABILITY OF THE KIT

- Unopened test kits should be stored at 2-8ºC upon receipt. All reagents are stable through the expiration date printed on the box. Return reagents to 2-8ºC immediately after use.
- Allow the sealed pouch to reach room temperature before opening the pouch and removing the required number of strips to prevent condensation of the microwell plate. The remaining unused strips should be stored in the original resealable pouch at 2-8ºC and can be used within 1 month of the opening date.
- Do not expose reagents especially the Substrate to strong light or hypochlorite fumes during storage or incubation steps.

SAMPLE (SPECIMEN) COLLECTION AND HANDLING

- The **BeneSphera™ T3** can be performed using only human serum collected from venipuncture whole blood.
- Separate serum from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipidic or turbid samples should not be used. Specimen with extensive particulate should be clarified by centrifugation prior to use. Do not use samples with fibrin particles or contaminated with microbial growth.
- Do not leave specimens at room temperature for prolonged periods. Serum specimens may be stored at 2-8ºC for up to 48 hours prior to assaying. For long term storage, specimens should be kept frozen below -20ºC.
- Bring specimens to room temperature prior to testing. Frozen specimens must be completely thawed and mixed well prior to testing. Specimens should not be frozen and thawed repeatedly.
- If specimens are to be shipped, they should be packed in compliance with local regulations covering the transportation of etiologic agents.

PREPARATION OF THE REAGENTS

1. All reagents should be brought to room temperature (21-25ºC) before use.
2. Adjust the incubator to 37ºC.
3. **Working Wash Buffer Solution:** Add 1 bag of PBS-T powder to 500 ml of distilled water, and mix well with magnetic stirrer. The Wash Solution is stable at room temperature for 2 months.
4. **Prepare Enzyme Conjugate Reagent:** add 0.1 ml of enzyme conjugate concentrate to 1.0 ml of enzyme conjugate diluent (1:10 dilution), and mix well. The amount of conjugate diluted is depending on your assay size. The conjugate reagent is stable at 4ºC for 7 days.

IMPORTANT NOTES

1. Do not use reagents after expiration date.
2. Do not mix or use components from kits with different lot numbers.
3. It is recommended that not more than 32 wells be used for each assay run, if manual pipette is used, since pipetting of all standards, specimens and controls should be completed within 5 minutes. A full plate of 96 wells may be used if automated pipette is available.
4. Replace caps on reagents immediately. Do not switch caps.
5. The wash procedure is critical. Insufficient washing will result in poor precision and invalid results.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder. Make data sheet with sample identification.
2. Dispense 50µl of calibrators, controls and samples into appropriate wells. Dispense 100µl of enzyme conjugate reagent into each well.
3. Thoroughly mix for 60 seconds. It is important to have complete mixing in this step.
4. Cover the plate and incubate at 37ºC for 60 minutes.
5. Remove the incubation mixture by flicking plate contents into a waste container.
6. Rinse and flick the microtiter plate 5 times with working wash solution.
7. Strike the wells sharply onto absorbent paper to remove residual water droplets.

Note: Improper washing may cause false results.

8. Dispense 50µl of Substrate A, than 50µl of Substrate B into each wells, gently mix for 10 seconds. Cover the microwell plate with the Plate Sealer or aluminum foil and incubate it at 21-25ºC for 5 minutes.
9. Read the relative light units (RLU) in each well using a microplate luminometer.The results should be read within 15 minutes of adding the working substrate solution.

CALCULATION OF RESULTS

1. Calculate the mean value from any duplicate reagents. Where appropriate, the mean values should be used for plotting.
2. On linear graph paper plot the RLU (ordinate) for each Reference Standard against the corresponding concentration of T3 in ng/ml (abscissa) and draw a calibration curve through the calibrator points by connecting the plotted points with straight lines.
3. Read the concentration for each control and sample by interpolating on the calibration curve.
4. Computer assisted data reduction will simplify these calculations. If automatic result processing is used, a 4-parameter logistic function curve fitting is recommended.

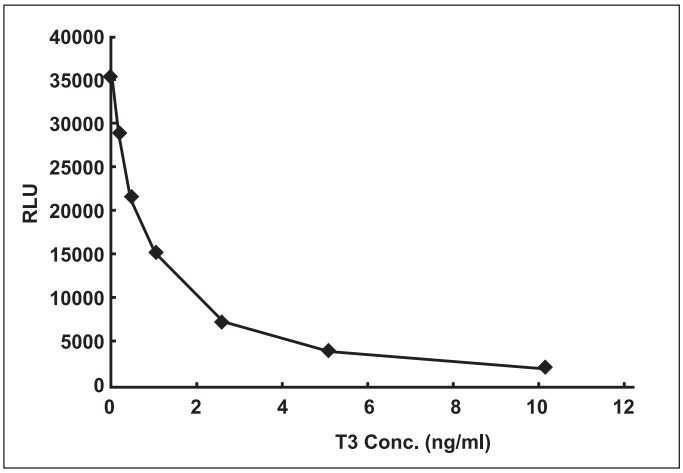
INTERPRETATION OF RESULTS

1. Results obtained as >>>>> sign signifies, obtained sample concentration is greater than calibrator F.

EXAMPLE OF STANDARD CURVE

A typical standard curve shown below is for the purpose of illustration only, and should never be used instead of the real time calibration curve.

	T3 (ng/ml)	RLUs
Calibrator A	0.00	35443.9
Calibrator B	0.50	21597.4
Calibrator C	1.00	15179.2
Calibrator D	2.50	7575.9
Calibrator E	5.00	4126.9
Calibrator F	10.00	2196.2



EXPECTED VALUES

Each laboratory should establish its own normal range. These values are given only for guidance.

Sample Numbers	147
Average Value (ng/ml)	1.37
Standard Deviation (σ)	0.27
Normal Range (± 2σ, ng/ml)	0.8-1.9

PERFORMANCE

A. Sensitivity

Twenty zero standards were assayed along with a set of other standards. The detection limit, defined as the apparent concentration corresponding to two standard deviations below the average RLU at zero binding, was lower than 0.2ng/ml.

B. Specificity

The cross-reactivity of the T3 assay with T4 and rT3 was determined by adding these interfering hormones to zero standards. The RLU produced was then determined.

Interferent	Concentration	Measured Value (ng/ml)	Crosstalk Rate (%)
T4	500ng/ml	0.44	<0.088
rT3	500ng/ml	0.26	<0.052

C. Precision

a. Infra-Assay Precision

Intra-Assay Precision was determined by assaying 20 replicates of each of 2 sera; low and high.

Serum	Number	Mean	SD	RSD (%)
Low	20	10.02	0.059	5.78
High	20	7.13	0.227	3.19

b. Inter-Assay Precision

Inter-assay Precision was determined by assaying duplicates of each of 2 serum pools in 20 separate runs, using a standard curve constructed for each run.

Serum	Number	Mean	SD	RSD (%)
Low	20	1.11	0.054	4.89
High	20	6.96	0.392	5.63

D. Accuracy

For 95 samples in the range of 0.4ng/ml to 8.5ng/ml, the relationship between the T3 CLIA Test and the Biocheck® T3 ELISA Test is described by the equation:

Method	Number Of Specimens	Least Square Regression Analysis	Correlation Coefficient
T3 CLIA	112	Y=0.9479X + 0.3762	0.954

LIMITATIONS

Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference thus anomalous values may be observed. Additional information may be required for diagnosis.

Serum T3 concentration is dependent upon a multiplicity of factors: hypothalamus gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of T3 to TBG. Thus, total triiodothyronine concentration alone is not sufficient to assess clinical status.

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