BeneSphera™

T4 Microwell CLIA (IMF)

INTENDED USE

The **BeneSphera™ T4 (IMF)** CLIA assay is designed for the quantitative determination of Thyroxine (T4) concentration in human serum.

INTRODUCTION

Thyroxine or 3, 5, 3'. 5'-tetraiodo-L-thyronine (T4) is the major hormone produced by the thyroid gland. It has a molecular weight of 777 daltons and is synthesized by iodination of tyrosine residues on thyroglobulin. Proteolytic cleavage of follicular thyroglobulin releases T4 into the bloodstream. Greater than 99% of T4 is reversibly bound to 3 plasma proteins in blood thyroxine binding globulin (TBG) binds 70%, thyroxine binding pre-albumin (TBPA) binds 20%, and albumin binds 10%. Approximately 0.03% of T4 is in the free, unbound state in blood at any time.

Diseases effecting thyroid function may present a wide array of confusing symptoms. Measurement of total T4 by immunoassay is the most reliable and convenient screening test available to determine the presence of thyroid disorders in patients. Increased levels of T4 have been found in hyper-thyroidism due to Grave's disease and Plummer's disease and in acute and subacute thyroiditis. Low levels of T4 have been associated with congenital hypothyroidism, myxedema, chronic thyroditis (Hashimoto's disease), and with some genetic abnormalities.

PRINCIPLE OF THE TEST

In the T4 CLIA, a certain amount of anti-T4 antibody is coated on microtiter wells. A measured amount of patient serum, and a constant amount of T4 conjugated with horseradish peroxidase are added to the microtiter wells, 8-anilino-1-napthalene sulfonate (ANS) is used to displace T4 from proteins to enable the measurement of total circulating T4. During incubation, T4 and conjugated T4 compete for the limited binding sites on the antiT4 antibody. After 60 minutes incubation at 37ºC, 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) of the reaction is inversely related to the concentration of T4 in the test sample.

KIT CONTENTS

1. **Coated Wells-** 1

   Microplate with anti T4 antibody coated wells.

2. **Calibrators-** 2A - 2F

   Six bottles of human serum reference containing the following concentration of T4 with preservatives.

   (A) Calibrator 0.0 µg/dl (B) Calibrator 1.0 µg/dl
   (C) Calibrator 2.5 µg/dl (D) Calibrator 5.0 µg/dl
   (E) Calibrator 15.0 µg/dl (F) Calibrator 30.0 µg/dl

3. **Enzyme Conjugate:** 3

   Horseradish Peroxidase (HRP) labeled anti-T4 in Stabilizing Buffer.

4. **Wash Buffer Concentrate / PBS-T Powder:** 4

   PBS-Tween

5. **Substrate A** 5A

   The reagent contains LuminGlo chemiluminescent substrate in 2.0% Dimethyl sulfoxide.

6. **Substrate B** 5B

   The reagent contains LuminGlo chemiluminescent substrate buffer with 30% hydrogen peroxide.

7. **Insert - 01 No.**
8. **Microwell Plate Cover**
9. **Zip Seal Polybag**

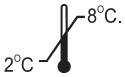
MATERIALS REQUIRED (BUT NOT PROVIDED)

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 product 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™ T4** 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 REAGENT

1. All reagents should be brought to room temperature (21-25°C) before use.
2. Adjust the incubator to 37°C.
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.

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 37°C 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 T4 in µ g/dl (abscissa) and draw a calibration curve through the reference standard 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.

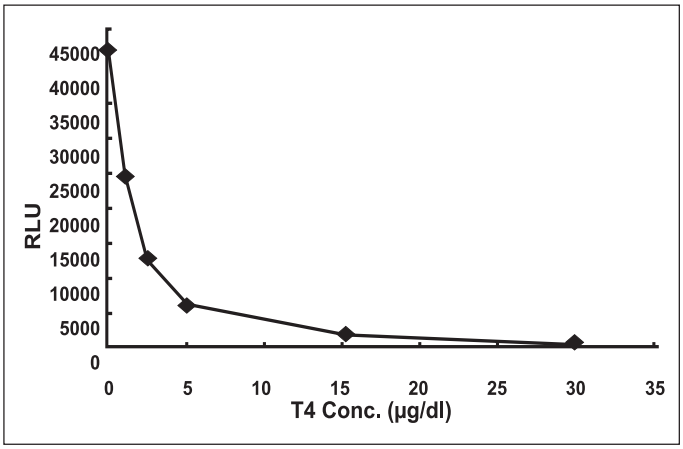
EXAMPLE OF STANDARD CURVE

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.

	T4 (µg/dl)	RLUs
Calibrator A	0.00	42572.9
Calibrator B	1.00	24617.2
Calibrator C	2.50	12632.5
Calibrator D	5.00	5783.5
Calibrator E	15.00	1643.1
Calibrator F	30.00	694.8

EXPECTED VALUES

A normal range of 5 µg/dl to 13 µg/dl (central 95% interval) was obtained by testing serum specimens from 187 individuals determined as normal by TSH CLIA kit and Total Thyroxine (T4) CLIA kit. It is recommended that each laboratory establish its own normal range



PERFORMANCE

A. Sensitivity

Twenty zero standards were assayed along with a set of other standards. The sensitivity, defined as the apparent concentration corresponding to two standard deviations below the average RLU at zero binding, was lower than 0.4 µg/dl.

B. Specificity

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

Interferent	Concentration	Measured Value (µg/dl)	Crosstalk Rate (%)
T3	500ng/ml	1.08	2.16
rT3	500ng/ml	0.78	1.56

C. Precision

a. Intra-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	3.94	0.23	5.83
High	20	11.23	0.42	3.74

b. Inter-Assay Precision

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

Serum	Number	Mean	SD	RSD (%)
Low	20	3.87	0.33	8.53
High	20	10.85	1.05	9.68

D. Accuracy

For 90 samples in the range of 1.5 µg/dl to 25 µg/dl. the relationship between the T4 CLIA Test and the Biocheck® T4 ELISA Test is described by the equation below:

Method	Number Of Specimens	Least Square Regression Analysis	Correlation Coefficient
T4 CLIA	127	Y=1.321X + 0.9367	0.957

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.

Serum T4 concentration is dependent upon a multiplicity of factors: hypothalamus gland function and its regulation, thyroxine binding globulin (TBG) concentration, and the binding of T4 to TBG. Thus, total T4 concentration alone is not sufficient to assess clinical status.

Heterophilic antibodies in human serum can react with reagent immunoglobulin, 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.

For diagnostic purposes, the results obtained form this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

QUALITY CONTROL

Good laboratory practice requires that quality control specimens be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

REFERENCES

1. Young DS. Effects of drugs on clinical laboratory tests. Ann. Clin. Biochem. 1997-34 (Pt 6):579-581.
2. Sterling K. Diagnosis and treatment of thyroid diseases. Cleveland: CRC Press; 1975.
3. Surks MI, Chopra IJ, Mariash CN, Nicoloff JT, Solomon DH. American Thyroid Association guidelines for use of laboratory tests in thyroid disorders. JAMA. 1990;263(11):1529-1532.
4. Glinoer D. The Regulation of Thyroid Function in Pregnancy: Pathways of Endocrine Adaptation from Physiology to Pathology. Endocr Rev. 1997;18(3):404-433.
5. Demers LM, Spencer CA. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Clin. Endocrinol. (Oxfi. 2003;58(2):138-140.

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