BeneSphera™

T3 Microwell ELISA

Enzyme Immunoassay for the quantitative determination of T3 hormone concentration in human serum/plasma. (*for In vitro* diagnostic use only)

SUMMARY:

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 activity and the development of the skeletal and organ system.

The determination of T3 levels in serum is essential in assessing thyroid functions. In most hyperthyroid patients, both serum T3 and T4 are elevated. However a condition known as T3 thyrotoxicosis exhibits only elevated T3 concentrations. Serum T3 levels are also an excellent indicator of the effectiveness of thyroid therapy.

TEST PRINCIPLE:

Competitive EIA (Quantitative)

In a competitive EIA, there exists a competitive reaction between native antigen and enzyme antigen conjugate for a limited number of insolubilized binding sites on the antibody coated on the microwell. After the antigen, antibody reaction has taken place, the fraction of the antigen in the conjugate or native antigen from the sample, which does not bind to the coated well, is washed away. The enzymatic activity in the antibody bound fraction, which is inversely proportional to the native antigen concentration, is measured by addition of the substrate.

By utilizing calibrators of known antigen values, a dose response curve can be generated from which the antigen concentration in a sample can be found out.

KIT CONTENTS:

1. **T3 Antibody Coated Microplate-** 1
   One 96-well microplate coated with Sheep anti-T3 serum and packaged in an aluminum bag with a drying agent.
2. **T3-Enzyme Conjugate (1.5ml/vial)-** 2
   One vial of T3-horseradish peroxidase (HRP) conjugate in an albumin-stabilizing matrix. A preservative has been added. Store at 2-8°C.
3. **T3 Conjugate Buffer (13ml)-** 3
   One bottle reagent containing buffer, red dye, preservative, and binding protein inhibitors. Store at 2-8°C.
4. **T3 calibrators (1 ml/vial) -** 4A - 4F
   Six vials of serum reference for triiodothyronine at concentrations of 0 4A, 0.5 4B, 1.0 4C, 2.5 4D, 5.0 4E and 7.5 4F ng/ml. Store at 2-8°C. A preservative has been added.
5. **Substrate A (7 ml/vial)-** 5
   One bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.
6. **Substrates (7 ml/vial)-** 6
   One bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.
7. **Wash Solution Concentrate (20 ml)-** 7
   One vial containing a surfactant in phosphate buffered saline. A preservative has been added. Store at 2-30°C.
8. **Stop Solution (8ml/vial) -** 8
   One bottle of stop solution containing a strong acid (1N HCl). Store at 2-30°C.
9. **Product Insert -** 01 No.

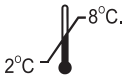
MATERIAL, TOOLS AND EQUIPMENT REQUIRED:

1. Micro plate reader with 450 nm and 620 nm wave length absorbance capability (the 620 nm filter is optional).
2. Micro plate washer or a squeeze bottle (optional).
3. Quality control material.
4. Timer.
5. Micro plate cover for incubation steps
6. Storage container for storage of wash buffer.
7. Vacuum aspirator (optional) for wash steps.
8. Deionized water.
9. Pipettes capable of delivering 50 µl and 100 µl.
10. Dispenser(s) for repetitive deliveries of 0.1 ml and 0.3 ml volumes with a precision of better than 1.5% (optional).
11. Containers for mixing reagents.

PRECAUTIONS: ⚠

1. Reagents are for *in vitro* diagnostic use only IVD.
2. Please handle all reagents and controls provided in the kit as potentially infectious although they are non reactive for HIV 1 and 2, HBsAg and HCV by FDA approved tests.
3. The stop solution provided in the kit is a strong acid (1 N HCl). Please wear gloves and face mask to avoid contact with the skin.
4. Please use disposable container or acid washed tubes (Washed with 1 N HCl or IN H_2,SO_4, rinsed well with deionised water and dried before use.) for preparing the substrate

PREPARATION OF REAGENTS AND STORAGE:



1. **Working conjugate solution**
   Prepare the working conjugate solution by mixing 160µl of conjugate concentrate with 1.6 ml of the conjugate buffer (1:11 ratio). This quantity of working solution is sufficient for two strips. The prepared reagent should be stored at 2-8°C and must be used within 24 hrs of preparation.
2. **Wash Solution**
   Prepare a 1:50 dilution of Wash Solution by mixing 1 ml of concentrated wash solution with 49 ml of distilled or deionised water. The prepared diluted wash solution can be preserved at 21 -25°C for 60 days.
   When stored at 2-8°C the wash solution concentrate may get crystalized, this is to be used only after thawing properly by keeping at 37°C for some time.
3. **Working Substrate Solution**
   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 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. Do not expose it to direct light).
   Note: Do not use the working substrate if it looks blue.

ASSAY PROTOCOL:

Before starting the assay, allow all the reagents serum references & controls to reach room temperature (21-25°C).

1. Format the micro plate wells for each calibrator and patient specimen to be assayed. Replace the unused micro well strips back into the aluminum foil, seal and store at 2-8°C.
2. Add 50 µl of the calibrator and the patient specimen to assigned wells.
3. Add 100 µl of the enzyme T3 reagent to each well. Care should be taken to dispense the entire reagent to the bottom of the coated well.
4. Shake the micro plate gently for 20-30 seconds to mix & cover.
5. Incubate for 60 min. at room temperature
6. Aspirate the contents of the wells & fill them completely (approximately 300 µl) with the diluted washing solution. Repeat the aspiration and washing procedure two times. After the last wash, blot the micro plate on some absorbent tissue to remove excess liquid from wells.
7. Add 100 µl of Working Substrate Solution to all the wells. Always add reagent in the same order to minimize reaction time difference between wells.
8. Incubate at room temperature (21-25°C) for fifteen (15) minutes.
9. Add 50 µl of Stop Solution to each well and mix well for 15-20 seconds.
10. Read the absorbance in each well at 450 nm (using a reference wave length of 620-630 nm to minimize well imperfections) in a micro plate reader

QUALITY PARAMETERS:

Every control with known concentration in hypothyroid, euthyroid and hyperthyroid range must be included in every run. Each laboratory must establish its own acceptable assay performance limits. Run to run reproducibility must be observed in a batch. If there is any deviation from the established data, it could be due to degradation in the kit components or change in the experimental conditions.

The absorbance of the calibrator 4A (0.0ng/ml) should be >1.3 for an assay to be valid.

GUIDELINES:

A. Performance of the Assay

1. Same sequence of reagent addition should be maintained throughout the run so that assay drift can be avoided.
2. Do not touch the bottom of the wells.
3. Improper washing may lead to faulty results.
4. Lipemic, hemolyzed and contaminated specimen should not be used.

B. Interpretation of results

1. The concentration obtained of the calibrators should be within ±10% of the assigned values in ng/ml.
2. Serum triiodothyronine concentration obtained is regulated by many factors like the thyroid gland function and its regulating, thyroxin binding globulin (TBG) concentration and the binding of triiodithyronine to TBG. Thus total triiodothyronine concentration alone is not sufficient to assess clinical status.
3. It has been observed that a decrease in total triiodithyronine is found in protein wasting diseases certain lever diseases and administration of testosterone, diphenylhydantoin orsalicylates.

NOT INTENDED FOR NEW BORN SCREENING

RANGE:

Aeuthyroid adult population was studied and the generalized range obtained is as given below.

Expected ranges :0.52-1.85 ng/ml

CALCULATION OF RESULTS:

A. Calculate the absorbance value of calibrators & samples at 450 nm. (Use 630 nm filter as reference filter, if available).
B. Plot a point to point curve by plotting the absorbance of each calibrator on y axis against concentration of each calibrator on the x axis.
C. Using the absorbance value for each sample determine the corresponding concentration of triiodothyronine in ng/ml. from the standard curve obtained.

PRECISION AND ACCURACY:

BeneSphera™ T3 was studied for intra assay & inter assay reproducibility by analysis on pooled control area.

Intra Assay reproducibility:

3 sets of pooled samples (10 in each) were studied for intra assay reproducibility and for low value samples it was found to be 8.9% while for normal & high value samples it was < 5.5%.

Inter Assay reproducibility:

3 sets pooled samples (10 in each) were studied for inter assay reproducibility and for low value samples a CV of 8.9 % was obtained while for normal & high value samples, CV obtained was < 7 %.

ACCURACY:

BeneSphera™ T3 results were compared with a reference radio immunoassay method. Totally 162 samples were used from euthyroid, hypothyroid and hyperthyroid cases. (Range 0.15 ng/ml-8.0 ng/ml). The total no. of such specimens was 120.

Excellent method agreement was found between the two methods.

SENSITIVITY:

BeneSphera™ T3 offers the sensitivity of 0.04 ng/ml.

SPECIFICITY:

The extent of cross reactivity of the T3 antibody to selected substances was evaluated by adding the interfering substances was found to be negligible.

BIBLIOGRAPHY:

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