BeneSphera™

TSH Microwell CLIA

INTENDED USE

Chemiluminescence Immunoassay (CLIA) for the Quantitative determination of thyroid stimulating hormone (TSH) in Human Serum or plasma.

INTRODUCTION

The determination of serum or plasma levels of thyroid stimulating hormone (TSH) is recognized as a sensitive method in the diagnosis of primary and secondary hypothyroidism. TSH is secreted by the anterior lobe of the pituitary gland and induces the production and release of thyroxine and triiodothyronine from the thyroid gland. It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta. Although the concentration of TSH in the blood is extremely low, it is essential for the maintenance of normal thyroid function. The release of TSH is regulated by a TSH-releasing hormone (TRH) produced by the hypothalamus. The levels of TSH and TRH are inversely related to the level of thyroid hormone. When there is a high level of thyroid hormone in the blood, less TRH is released by the hypothalamus, so less TSH is secreted by the pituitary. The opposite action will occur when there is decreased thyroid hormone in the blood. This process is known as a negative feedback mechanism and is responsible for maintaining the proper blood levels of these hormones. TSH and the pituitary glycoproteins: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG), have identical alpha chains. The beta chain is distinct but does contain identical amino acid sequences, which can cause considerable cross-reactivity with some polyclonal TSH antisera. The use of a monoclonal antibody in this TSH EIA test eliminates this interference, which could result in falsely elevated TSH values in either menopausal or pregnant females, a population whose evaluation of thyroid status is clinically significant.

PRINCIPLE OF THE KIT

BeneSphera™ TSH is based on the principle of a solid phase enzyme-linked immunosorbent assay. The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies with distinct epitope recognition. In this procedure, the immobilization takes place during the assay at the surface of a microwell plate through the interaction of streptavidin coated on the wells and exogenously added biotinylated monoclonal anti-TSH antibody.

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 CONTENETS

1. **Microwell plate-** 1

   White microplate wells coated with streptavidin (500 ng/well and blocked with 1.0 % Bovine serum Albumin).

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

   Six bottles of serum reference containing the following amounts of Thyroid stimulating hormone (TSH), Preservative: 0.2% Proclin 300 (5-Chloro-2 methyl-1, 2-thiazol-3-one).

   (A) Calibrator 0.0 µIU/ml, (B) Calibrator 0.5 µIU/ml,

   (C) Calibrator 2.5 µIU/ml, (D) Calibrator 10.0 µIU/ml,

   (E) Calibrator 20.0 µIU/ml, (F) Calibrator 40.0 µIU/ml,

3. **TSH Tracer reagent -** 3

   This reagent containing anti-h TSH (TSH Horseradish Peroxidase) conjugates in an albumin stabilizing matrix, Preservative: 0.2% Proclin 300 (5-Chloro-2 methyl-1, 2-thiazol-3-one).

4. **Substrate A-** 4A

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

5. **Substrate B-** 4B

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

6. **Wash Buffer concentrate-** 5

   10x concentration, Phosphate buffer saline containing 8.0% Sodium Chloride, 0.2% Potassium Chloride, 1.4% di Sodium Hydrogen Orthophosphate, 0.24% Potassium di-Hydrogen orthophosphate, 0.1% Tween 20 (Polyoxyethylene (20) sorbitan monolaurate.

7. **Package Insert** (1 No.)

MATERIALS REQUIRED (BUT NOT PROVIDED)

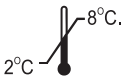
1. Pipette capable of delivering 10 µl to 100 µl and 100 µl to 1000 µl
2. Disposable pipette tips
3. Microplate washer
4. Microplate luminometer
5. Absorbent paper for blotting the microplate wells
6. Microplate sealer or aluminum foil for incubation steps
7. Timer
8. Quality control materials
9. Deionised water or purified water
10. Clean containers for mixing of reagents
11. 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 microplate 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.
- 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 Conjugate 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.
- Avoid any contact of the Substrate A and Substrate B with skin or mucosa.
- Non-disposable apparatus should be sterilized after use. The preferred method is to autoclave for one hour at 121°C. Disposables should be autoclaved or incinerated. Do not autoclave materials containing sodium hypochlorite.
- 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 2-8°C upon receipt. All reagents are stable through the expiration date printed on the box. Return reagents to 2-8°C 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 2-8°C and can be used within 1 month of the opening date.
- Concentrated Wash Buffer may be stored at room temperature to avoid crystallization. If crystals are present, warm up the solution at 37°C. Working Wash Buffer is stable for 2 weeks at room temperature.
- 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™ TSH** can be performed using only human serum or plasma collected from venipuncture whole blood.
- EDTA, heparin, and ACD collection tubes may be used to collect venipuncture whole blood and plasma specimens. The preservative sodium azide inactivates horseradish peroxide and may lead to erroneous results.
- Separate serum or plasma from blood as soon as possible to avoid hemolysis. Grossly hemolytic, lipemic or turbid samples should not be used. Samples 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 and plasma samples may be stored at 2-8°C for up to 48 hours prior to assaying. For long term (but not more than thirty days) storage, specimens should be kept frozen below -20°C.
- 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. **Wash Buffer**

   Prepare Working Wash Buffer by diluting the Concentrated Wash Buffer 1:10. Pour 10 ml of the concentrated wash buffer in a 100 ml graduated cylinder and fill it with freshly purified or deionised water to 100 ml. It is stable for 2 weeks at 21-25°C.

   Note: If crystals are present in the Concentrated Wash Buffer, warm it up at 37°C until all crystals dissolve.

2. **Working Substrate Solution**

   Determine the amount of the reagent needed and prepare by mixing equal portion of substrate A and substrate B in a clean and dried container. Keep the solution in dark. The reagent should be used within 12 hours, if stored at 2-8°C.

ASSAY PROCEDURE

1. Allow all the reagents and samples should reach to room temperature (21-25°C) prior to testing. The procedure must be strictly followed. Assay must proceed to completion within time limits.
2. Format the microplate well for each serum calibrator, control and sample to be assayed. Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.
3. Add 10 µl of each calibrator in wells A1 and F1.
4. Add 10 µl of controls and samples to assigned wells starting at G1.
5. Add 50 µl of TSH Tracer reagent to each well starting from A1.
6. Mix gently by swirling the microwell plate on a flat bench for 1 minute. Cover the microwell plate with the Plate Sealer or aluminum foil and incubate it at 21-25°C for 30 minutes.
7. Remove the Plate Sealer or aluminum foil and wash each well 5 times with 300 µl of Working Wash Buffer per well, then remove the liquid. Turn the microwell plate upside down on absorbent tissue for a few seconds. Ensure that all wells have been completely washed and dried.

Note: Improper washing may cause false results.

8. Add 100 µl of working substrate solution to each well. Mix gently by swirling the microwell plate on a flat bench for 1 minute. Cover the microwell plate with the Plate Sealer or aluminum foil and incubate it at 21-25°C 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

A. Calculate the RLU of calibrators, control and sample with the help of luminometer.

B. Plot a point to point curve by plotting the RLU of each calibrator on Y axis against concentration of each calibrator on X axis.

C. Using the RLU for each control and sample determine the corresponding concentration of TSH in µIU/ml from the standard curve obtained.

INTERPRETATION OF RESULTS

1. The concentration obtained of the calibrators should be within ± 10% of the assigned values in µIU/ml.
2. Serum TSH concentration is dependent upon a multiplicity of factors such as hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. Thus,

thyrotropin concentration alone is not sufficient to assess clinical status.

3. Serum TSH values may be elevated by pharmacological intervention. Domperiodone, amiodazon, iodide, Phenobarbital, and phenytoin have been reported to increase TSH levels.
4. A decrease in thyrotropin values has been reported with the administration propanolol, methimazol, dopamine and d-thyroxine.
5. Genetic variation or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results among various assay systems due to the antibodies involved.

NOT INTENDED FOR NEW BORN SCREENING

QUALITY CONTROL AND PARAMETERS

1. The controls 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 each 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.
2. The dose response curve should be within established parameters.
3. Four out of six controls should be within the established range.

EXPECTED RANGES FOR SAMPLES

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

Expected range for samples: 0.42 µIU/ml - 5.45 µIU/ml

LIMITATIONS OF PROCEDURE

1. It is important that the time of reaction in each well is held constant for reproducible results.
2. Pipetting of calibrators, samples and controls should not extend beyond 10 minutes to avoid assay drift.
3. Dose response curve should be repeated after every run.
4. The improper aspiration or decantation wash steps may cause in poor and spurious results.
5. Multichannel pipettes are recommended for addition of reagents.

PERFORMANCE CHARACTERISTICS

1. Precision

The intra assay precisions of the assay were determined for the **BeneSphera™ TSH** by analyses on three different levels of samples. The mean value, standard deviation and coefficient of variation for each sample were analyzed and presented in below mentioned table.

Intra Assay			
Level	**X**	**STDV**	**CV**
Level 1	2.9	0.2	7.5
Level 2	28.9	2.1	7.4
Level 3	44.6	3.2	7.2

The inter assay precision of the assay were determined by analyses of samples in 10 different assay. The mean value, standard deviation and coefficient of variation for sample was analyzed and presented in below mentioned table.

Inter Assay	
Value	25.402
STDV	1.183
CV	4.6

2. Accuracy

Ability of the assay to match the value of the clinical sample being measured. The **BeneSphera™ TSH** were compared with a reference Cobas (Roche system). Least square regression equation and correlation coefficient were computed for the **BeneSphera™ TSH** and reference method.

Metric	TSH
Correlation Coefficient	98%
Limit of agreement	1.24 to -1.84

The **BeneSphera™ TSH** has shown excellent correlation with the sample prediction on fully automated Cobas (Roche) system.

3. Sensitivity

The sensitivity (detection limit) was ascertained by determining the variability of the 0.0 µIU/ml **BeneSphera™ TSH** serum calibrator and using the 2σ (95% certainty) to calculate the minimum dose. The **BeneSphera™ TSH** has a sensitivity of 0.03 µIU/ml.

4. Specificity

The extent of cross reactivity of the **BeneSphera™ TSH** to selected substances was evaluated by adding the interfering substance was found to be negligible.

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