BeneSphera™

TSH Microwell ELISA

The human thyroid stimulating hormone (hTSH) ELISA system provides a direct quantitative determination of hTSH in human serum. hTSH can be assayed in the range of 0.39-6.16 µIU/ml using 50µl serum samples.

SUMMARY:

The Thyroid Stimulating Hormone (thyrotropin or TSH) is a glycoprotein with a molecular weight of 28000, secreted by the adenohypophysis. TSH is controlled by negative feedback from circulating T3, T4 and TRH (Thyrotropin Releasing Hormone).

The measurement of serum TSH has proven to be one of the most sensitive methods for the detection of primary hypothyroidism. In primary hypothyroidism the production of thyroid hormones is impaired and the TSH levels are observed to be higher. However, in secondary and tertiary hypothyroidism, the TSH levels are low because of pituitary or hypothalamic lesions.

In hyperthyroidism the circulating level of TSH is usually subnormal. In some instances, however this condition may result from hyperstimulation of the thyroid, due to hypothalamic or pituitary lesions and in this case the TSH level is increased.

Measurement of circulating levels of TSH may be used as a screening or confirmatory test tor hypothyroidism and hyperthyroidism, and as an aid to differential diagnosis of these conditions. TSH levels have been used to monitor the adequacy of thyroid hormone replacement therapy.

TEST PRINCIPLE:

Quantitative EIA

In a quantitative EIA, high affinity antibodies react with antigen to form an insoluble sandwich complex on the surface of a coated microplate. The antigen from the specimen gets linked at the surface of the well through interaction of reactive IgG coated on the well and affinity purified x-antigen IgG conjugated with HRP. The fraction of the x-antigen IgG conjugated with enzyme that does not bind to the coated well is washed away. The enzyme activity, which is proportional to antigen concentration in the sample, is measured by addition of 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. **TSH Reactive Coated Microplate (96 wells) -** [1]
   One 96-well microplate coated with reactive IgG and packed in an aluminium bag with a drying agent. Store at 2-8°C
2. **Enzyme-TSH Reagent (13 ml/vial) -** [2]
   One vial containing 1HRP labeled, reactive x-TSH IgG, in buffer, dye, and preservative. Store at 2-8°C.
3. **Thyrotropin Calibrators (1 ml/vial) -** [3A] - [3G]
   Seven vials of references for TSH Antigen at levels of 0 [3A], 0.5 [3B], 2.5 [3C], 5.0 [3D] ,10 [3E] , 20 [3F] and 40 [3G] µIU/ml. Store at 2-8°C. A preservative has been added.
   Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IRP 80/558.
4. **Substrate A (7ml/vial)-** [4]
   One bottle containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.
5. **Substrate B (7ml/vial)-** [5]
   One bottle containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.
6. **Wash Solution Concentrate (20 ml)-** [6]
   One vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-30°C.
7. **Stop Solution (8ml/vial)-** [7]
   One bottle containing a strong acid (1N HCl). Store at 2-30°C.
8. **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. Absorbent paper for blotting the microplate.
4. Quality control material.
5. Timer.
6. Micro plate cover for incubation steps.
7. Storage container for storage of wash buffer.
8. Vacuum aspirator (optional) for wash steps.
9. Deionized water.
10. Pipettes capable of delivering 50 µl and 100 µl.
11. Dispensers) for repetitive deliveries of 0.1 ml and 0.3 ml volumes with a precision of better than 1.5% (optional).
12. Containers for mixing reagents.

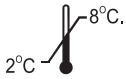
PRECAUTIONS: ⚠

1. Reagents are for *in vitro* diagnostic use only.
2. Please handle all reagents and controls provided in the kit as potentially infectious although they have been rendered infectious, and 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.

Please use disposable container or acid washed tubes for preparing the substrate (Washed with 1 N HCl or 1 N H_2SO_4, rinsed well with deionised water and dried before use.)

SAMPLE COLLECTION AND PREPARATION:

Serum or Plasma may be used for the test. Serum or plasma should be prepared from a whole blood specimen obtained by approved aseptic technique. If testing cannot be done within an hour after sample collection, refrigerate (maximum 48 hours) the specimen immediately and let it return.



PREPARATION OF REAGENTS AND STORAGE:

1. **Wash Solution**
   Prepare a 1:50 dilution of Wash Solution by mixing 1 ml of concentrated wash solution to 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, use that only after thawing properly by keeping at 37°C for some time.
2. **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).
   Note: Do not use the working substrate if it looks blue.

ASSAY PROTOCOL:

Before starting of the assay, allow all the reagents. Calibrators and serum samples 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 aluminium foil, seal and store it at 2-8°C.
2. Add 0.050 ml (50 µl) of the calibrator and the patient specimen to the assigned wells.
3. Add 0.100 ml (100 µl) of the Enzyme TSH Reagent to each well. Care should be taken to dispense all the reagents close to bottom of coated wells.
4. Shake the micro plate gently for 20-30 seconds to mix.
5. Incubate for 60 minutes at room temperature.
6. Aspirate the contents of the wells and fill them completely (approximately 300 µl) with the diluted washing solution. Repeat the aspiration and washing procedure for two more times. After the last wash, blot the micro plate on absorbent tissue to remove excess liquid from wells.
7. Add 0.100 ml (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 0.050 ml (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 should let 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 condition.

The absorbance of the calibrator 3G (40 µlU/ml) should be >1.3 for an assay to be valid.

CALCULATION OF RESULTS:

A. Calculate the absorbance value of calibrator & 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 x axis.
C. Using the absorbance value for each sample determine the corresponding concentration of hTSH in µlU/ml. Using the absorbance value for each sample, determine the corresponding/ml from the standard curve obtained as given above.

GUIDELINES:

A. Performance of the assay

1. Same sequence of reagent addition should be maintained throughout the test, so that assay drift can be avoided.
2. Don't 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 obtained concentration of the calibrators should be within ±10% of the assigned concentration.
2. Serum hTSH concentration obtained is regulated by many factors like the receptiveness of pituitary to TRH, hypothalamus gland functions etc. This concentration of hTSH alone is not sufficient to know the clinical status of a sample.
3. A decrease in serum hTSH concentration is observed with administration of propenolol, methimazol etc. Similarly an increase in serum hTSH concentration is observed by pharmacological intervention, domperiodone etc.
4. Any genetic variation or degradation of intact TSH into sub units affect the binding characteristic of the antibodies and may affect the final result.

Not intended for NEW BORN SCREENING.

RANGE:

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

Low Normal Range	0.39 µlU/ml
High normal range	6.16 µlU/ml

PRECISION AND ACCURACY:

BeneSphera™ TSH was studied for intra assay and interassay reproducibility by analysis on pooled control sera. The number of samples, mean value, standard deviation and C V of each of these control sera gave were as follows.

Intra Assay Reproducibility:
(values in µlU/ml)
3 sets pooled samples (24 in each) were studied for intra assay reproducibility and gave a coefficient of variation of <5% in all the three cases.

Inter Assay Reproducibility:
(values in µlU/ml)
3 sets pooled samples (10 in each) were studied for inter assay reproducibility and gave a coefficient of variation of <6%.

ACCURACY:

BeneSphera™ TSH results were compared with a reference immunochemiluminescence assay. Total 170 samples from euthyroid, hypothyroid and hyperthyroid samples were used (Range 0.01 µlU/ml-41 µlU/ml). The correlation coefficient and least square regression equation were computed for **BeneSphera™ TSH** in comparison with the reference method.

A very high degree of correlation was found between the **BeneSphera™ TSH** and reference immunochemiluminescence assay.

SENSITIVITY AND SPECIFICITY:

Sensitivity

BeneSphera™ TSH offers the following sensitivity.

One hour incubation procedure 0.078 µlU/ml.
Two hour incubation procedure 0.027 µlU/ml.

Specificity

The extent of cross reactivity of **BeneSphera™ TSH** with interfering substances was found to be negligible (<0.01% increase in the OD obtained with TSH) with 1000ng/ml of follitropin, lutropin and hCG.

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