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**ELISA** **E**NZYME **L**INKED **I**MMUNO**S**ORBENT **A**SSAY

## Microwell Method

# THYROTROPIN (TSH)

**Cat. No. Z01237**

*For in vitro Diagnostic Use*

P r o d u c t  I n s e r t

Enzyme Linked Immunosorbent Assay for the **quantitative** determination of Thyrotropin (Thyroid Stimulating Hormone) in human serum.

CE IVD

Microwell Method - 96 wells

(12 x 8-well Antibody coated Strips)

Individual breakaway

## INTENDED USE

The TSH ELISA is intended to be used for the quantitative determination of thyroid-stimulating hormone (TSH, thyrotropin) in serum.

## Clinical background

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28000 daltons and a secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism (1,2). Increase in serum concentrations of TSH, which is primarily responsible for the synthesis and release of thyroid hormones, is an early and sensitive indicator of decrease thyroid reserve and in conjunction with decreased thyroxine (T4) concentrations si diagnostic of primary hypothyroidism. The expected increase in TSH concentrations demonstrates the classical negative feedback system between the pituitary and thyroid glands That is, primary thyroid gland failure reduces secretion of the thyroid hormones, which in turn stimulates the release of TSH from the pituitary.

Additional, TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduces the response of the pituitary to the stimulatory effects of TRH. In secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH levels are generally low or normal. Either pituitary TSH deficiency (secondary hypothyroidism) or insufficiency of stimulation of the pituitary by TRH (tertiary hypothyroidism) causes this. The TRH stimulation test differentiates these conditions. In secondary hypothyroidism, TSH response to TRH is blunted while a normal or delayed response is obtained in tertiary hypothyroidsm.

Further, the advent of immunoenzymometric assays has provided the laboratory with sufficient sensitivity to enable the differentiating of hyperthyroidism from euthyroid population and extending the usefulness of TSH measurements. This method is a second-generation assay, which provides the means for discrimination in the hyper-thyroid-euthyroid range.

In this method, TSH calibrator, patient specimen or control is first added to an anti-TSH coated well. Monoclonal, enzyme labeled antibodies are added and the reactants mixed. Reaction between the various TSH antibodies and native TSH forms a sandwich complex that binds with the anti-TSH coated well.

After the completion of the required incubation period, the antibody bound enzyme-thyrotropin conjugate is separated from the unbound enzyme-thyrotropin conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known thyrotropin levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen’s activity can be correlated with thyrotropin concentration.

## PRINCIPLE OF TEST

The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies, with different and distinct epitope recognition, in excess and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of anti-TSH coated on the well and exogenously added monoclonal anti-TSH antibody.

Upon mixing a reaction takes place between fixed monoclonal antibody, the enzyme-labeled second antibody and a serum containing the native antigen. The reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a sandwich complex.
After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration.
The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration for an unknown can be ascertained.

**CONTENTS OF KIT**
Reagents are sufficient for 96 wells.

1. **Microwell plate:** 96 wells coated with TSH antibody, packed in a laminate bag. Ready for use.

2. **Calibrators:** Ready for use. Calibrators A-F 0.4 mL. The Calibrator values are approx. 0, 0.25, 0.75, 2.0, 5.0 and 15 mIU/L. Exact Calibrator values are given on the label of each vial.

3. **Control:** 0.4 mL. Ready to use. Concentration stated on the label.

4. **Enzyme Conjugate:** 12 mL. Ready to use.

5. **Wash Buffer:** 40 x concentrate, 25 mL. Before use dilute to 1000 ml with distilled water. Store at room temperature.

6. **Substrate Solution:** 12 mL, ready for use, contains TMB (Tetra-methylbenzidine)

7. **Stop Solution:** 12 mL, 0.25 mol/l $H_2SO_4$. Avoid contact with eyes and skin.

**MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED**
- Pipette with disposable plastic tips: 100 µL + 25 µL
- Multichannel pipette with disposable plastic tips: 50 µL, 100 µL (optional)
- Lid or sealing tape for microwell plate
- Reagent troughs
- Aspiration device, Washer (optional)
- Photometer (plate or strip reader) , 450 nm

**PRECAUTIONS**
All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control/ National Institute of Health, “Biosafety in Microbiological and Biomedical Laboratories”, 2nd Edition, 1988, HHS.

## SPECIMEN COLLECTION AND HANDLING

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain red-top venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2–8°C for a maximum period of five (5) days. If the specimen(s) can not be assayed within this time, the sample(s) may be stored at temperatures of –20°C for up to 30 days. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 mL of the specimen is required.

## STORAGE AND STABILITY.

The kit should be stored at 2–8°C. The unopened kit is stable until the expiry date printed on the kit label. The expiry date of each unopened component is printed on the label of the component.

## TEST PROCEDURE

**Note:** Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator. Condensation will adversely effect coating and will lead to false result.

1. Allow all reagents to reach room **temperature (20–30°C)** before use. Dilute the Wash Buffer.
2. Mark the wells to be used on the plate.
3. Pipette 25 µL of Calibrators, Control and serum samples into appropriate wells.
4. Incubate 10 minutes at room temperature.
5. Add 100 µL of Enzyme Conjugate to each well and swirl gently for 10 seconds.
***6. Cover the plate and incubate for 90 minutes at room temperature.***
7. Aspirate and wash the wells 5 times with 300 µL of wash buffer.
8. At timed intervals add 100 µL of Substrate Solution into each well.
***9. Cover the plate and incubate for 20 minutes at room temperature (20–25°C).***
10. Stop the reaction by adding 100 µL of Stop Solution into each well at the same timed intervals as in step 8. Shake the plate gently to mix the solutions.
11. Measure the absorbance at 450 nm using a plate or strip reader. The results should be read within five minutes after adding the stop solution. Preferably readings should take place immediately after stopping the reaction since the OD450 may slightly decrease with the course of time.

## NOTES

1. Protect the plates from draught, strong light or direct sunlight during the test procedure.
2. Careful aspiration of the wash buffer is essential for good assay precision.
3. Since timing of the incubation steps is important to performance of the assay, pipette the samples and the enzyme conjugate without interruption. If more than one plate is used in the same run it is recommended to include a Calibrator curve on each plate.

4. Adding of the Substrate Solution starts a kinetic reaction that is terminated by dispensing the Stop Solution. Keep the incubation times for each well the same by adding reagents at timed intervals.
5. Protected from light, absorbance values are stable for 60 minutes.
6. Plate readers measure absorbance vertically. Do not touch the bottoms of the wells.

**Results**

1. A dose response curve is used to ascertain the concentration of thyrotropin in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader.
2. Plot the absorbance for each duplicate serum reference versus the corresponding TSH concentration in mIU/L on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of TSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/L) from the horizontal axis of the graph (the duplicates of the unknown may be averaged).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction.
For low concentrations it is recommended to plot in a log-log or lin-lin scale.

**Q.C. PARAMETERS**

Maximum Absorbance (15 mIU/L) = 1.4–2.8

**LIMITATIONS OF PROCEDURE**

**A. Assay Performance**

It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
Sample(s), which are contaminated microbiologically, should not be used in the assay. Highly lipaemeic or haemolysed specimen(s) should similarly not be used.
Addition of the substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the addition of the substrate and the stopping solution should be added in the same sequence to eliminate any time deviation during reaction. Plate readers measure vertically. Do not touch the bottom of the wells.
Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
Patient specimens with TSH concentrations above 40 mIU/L may be diluted with the zero calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor. Each component in one assay should be of the same lot number and stored under identical conditions.

**Unused microwell strips should be inserted into the plastic bag provided with the kit inclusive the desiccant found inside the aluminum bag. Do not store unused strips in the opened aluminum bag since this may destroy the wells especially when stored for a longer time refrigerated (high moisture).**

### B. Interpretation

If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

Serum thyrotropin concentration is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. Thus, thyrotropin concentration alone is not sufficient to assess clinical status.

Serum thyrotropin values may be elevated by pharmacological intervention. Dom-periodone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.

A decrease in thyrotropin values has been reported with the administration of pro-pranolol, methimazol, dopamine and d-thyroxine (4).

Genetic variations 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 due to the reactivity of the antibodies involved.

## "NOT INTENDED FOR NEWBORN SCREENING"

## EXPECTED RANGES OF VALUES

A study of euthyroid adult population was undertaken to determine expected values for the TSH ELISA Microplate Test System. The number and determined range are given in Table 1. A nonparametric method (95% Percentile Estimate) was used.

**TABLE I**
**Expected Values for the TSH ELISA Test System**
**(in mIU/L)**

| | |
|---|---|
| Number | 139 |
| Low Normal Range | 0.39 |
| High Normal Range | 6.16 |
| 70% Confidence Intervals for 2.5 Percentile | |
| Low Range | 0.29 – 0.54 |
| High Range | 5.70 – 6.92 |

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an inhouse range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

## PERFORMANCE CHARACTERISTICS

### A. Precision

The within and between assay precision of the TSH ELISA were determined by analyses of three different levels of pool control sera. The number, mean value, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

**TABLE 2**

Within Assay Precision (Values in mIU/L )

| **Sample** | **N** | **X** | **S.D.** | **C.V.** |
|---|---|---|---|---|
| Pool 1 | 20 | 1,706 | 0,137 | 8,06% |
| Pool 2 | 20 | 7,662 | 0,459 | 5,99% |
| Pool 3 | 20 | 11,880 | 0,517 | 4,36% |

**TABLE 3**

Between Assay Precision* (Values in mIU/L)

| **Sample** | **N** | **X** | **S.D.** | **C.V.** |
|---|---|---|---|---|
| Pool 1 | 10 | 1,787 | 0,129 | 7,20% |
| Pool 3 | 10 | 11,981 | 1,021 | 8,52% |

*As measured in ten experiments in duplicate.

**B. Accuracy**

The Dialab TSH ELISA was compared with commercially available TSH assay. Biological specimens from 0.4 mIU/L to 12.3 mIU/L were used. The total number of such specimens was 41. The least square regression equation and the correlation coefficient were computed for the TSH ELISA in comparison with the reference assay. The data obtained is displayed in Table 4.

**TABLE 4**

| Method | Mean (x) | Least Square Regression Analysis | Correlation Coefficient |
|---|---|---|---|
| This Method | 5.08 | y = 1.02x - 0.63 | 0.985 |
| Reference | 5.60 | | |

Only slight amounts of bias between the TSH ELISA system and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

**C. Sensitivity**

The sensitivity (detection limit) was ascertained by determining the variability of the 0 mIU/L serum calibrator and using the $3\sigma$ statistic to calculate the minimum dose:
For 90 min incubation = 0.027 mIU/L.

**D. Specificity**

The relative percent cross-reactivity of the Dialab TSH ELISA method to selected substances was determined as a ratio of TSH concentration to the cross-reactive compound concentration needed to produce the same absorbance. The cross-reactivities are presented in Table 5.

**TABLE 5**

| Compound | Cross-Reactivity[%] |
|---|---|
| hFSH | 0.15 |
| hLH | 0.13 |
| hCG | 0.14 |

**E. Linearity**

A patient sample was serially diluted with zero standard in a linearity study. (Values in mIU/L ) The cross-reactivities are presented in Table 6.

**TABLE 6**

| Dilution | Expected Conc. | Observed Conc. | Recovery |
|---|---|---|---|
| | | 9.29 | |
| Dil. 1 / 2 | 4.65 | 4.99 | 107.5 % |
| Dil. 1 / 4 | 2.32 | 2.38 | 102.3 % |
| Dil. 1 / 8 | 1.16 | 1.09 | 94.2 % |

In this assay, no hook effect is observed up to 100 mIU/l of TSH.

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