TSH ELISA KIT

Enzyme-linked Immunosorbent Assay for Quantitative Determination of Thyroid -Stimulating Hormone in Human Serum

1. INTENDED USE

1.1. This kit is intended for quantitative determination of thyroid-stimulating hormone (TSH) in human serum.

1.2. TSH is a glycoprotein with a molecular weight of and beta. TSH is
secreted about30000Da, that consists of two subunits – alpha by frontal lobe of pituitary gland and stimulates thyroxin and triiodothyronine synthesis in thyroid gland. TSH assay is important in evaluation of thyroid status.

2. ASSAY DESCRIPTION AND PRINCIPLES

2.1. Kit Contents:

- microtitration strips, 12x8 wells, coated with anti-TSH monoclonal antibodies, packed in the plastic bag.

- TSH calibrators (protein-based buffer containing known TSH concentrations). For exact TSH Concentrations , see vial labels.

- anti-TSH antibodies conjugated with HRP.

- concentrated wash buffer.

- tetramethylbenzidine substrate solution.

- “Stop reagent”.

- control (protein-based buffer containing known TSH concentration) .

2.2. “ThyroidEIA-TSH” reagents are sufficient for determination of 40 unknowns, 6 calibrators, 1 control and 1 TMB Substrate control in duplicates, provided that all the strips are used simultaneously.

NOTE :if used partially, kit should be utilized within a month after opening.

2.3. Assay principle. “ThyroidEIA-TSH” is a “sandwich” type of solid-phase enzyme immuno-assay, based on two monoclonal antibodies specific for different determinants of TSH β-subunit. One of these antibodies is conjugated with horseradish peroxidase; the other is immobilized on inner surface of microwells. TSH molecules from the serum sample binds to both immobilized and conjugated antibodies. Then the wells are washed to remove any material not bound to the inner surface of the wells. The quantity of the bound conjugate is directly proportional to TSH concentration in the sample. When TMB substrate solution is added to the wells, enzymatic reaction leads to color development. The intensity of appearing

color is directly proportional to TSH concentration in serum sample. The intensity of color, i.e. the optical density of the solution in the wells is measured and TSH concentration in the samples is calculated using the calibration curve.

3. PERFORMANCE CHARACTERISTICS

3.1. Specificity. No cross-interaction of monoclonal antibodies against beta-TSH with LH, FSH and hCG was detected.

3.2. Coefficient of variation (intra-assay precision) between the results of TSH determination in the same sample is less than 8%.

3.3. Linearity (Dilution test). Dilution of serum sample containing predetermined TSH concentration with Calibrator№1 (0 μIU/ml) leads to linear recovery of TSH in diluted samples in concentration range between Calibrator №2 and Calibrator №6.

3.4. Recovery. To determine this parameter, equal volumes of control and Calibrator №3 were mixed. Then the correspondence between the calculated TSH concentration in the obtained sample and the measured concentration was determined. Recovery range is 90–110%.

3.5. Detectability. Minimal TSH concentration detectable by “ThyroidEIA-TSH” assay is 0.05 μIU/ml.

3.6. Expected values. Serum samples collected between 9 and 11 a.m. from 140 apparently healthy people (both males and females) at the age 21–45, were assayed with “ThyroidEIA-TSH” kit. TSH concentration range was 0.23–3.4 μIU/ml, mean 1.37 μIU/ml. These limits should be considered as guidelines only.

3.7. It is highly recommended for each laboratory to determine its own reference range of TSH concentrations.

4. WARNINGS AND PRECAUTIONS

4.1. All the components are non-toxic.

4.2. Stop reagent is 1N HCl solution. Avoid contacts with skin and mucosa. In case of contact rinse affected region thoroughly with plenty of water.

4.3. It is highly recommended to handle kit components in accordance with established good laboratory practice. The operator should wear disposable latex or plastic gloves and handle patients samples as if capable of transmitting infectious agents.

5. MATERIALS REQUIRED BUT NOT SUPPLIED WITH THE KIT:

- digital variable pipettes that cover volume range from 0.005 to 0.05 ml; from 0.04 to 0.2 ml; from 0.2 to 1 ml and from 1 to 5 ml, with appropriate disposable tips;

- 8-channel digital variable pipette that covers volume range up to 0.3 ml, with appropriate disposable tips;

- microplate shaker-thermostat , able to maintain temperature +37°C and shaking speed 500 to 800 rpm;

- automatic microplate reader;

- 200 ml volumetric cylinder;

- 300 ml volumetric beaker;

- distilled water.

6. REAGENT PREPARATION FOR ASSAY

6.1. TSH calibrators and control are ready to use. Once opened, store at +2… 8°C for no more than 1 month.

6.2. Preliminary dilution of serum samples. If expected TSH concentration in the samples are higher than in Calibrator №6, samples should be diluted 20-fold with Calibrator №1 (0 μIU/ml):

20 μl of serum sample + 380 μl of Calibrator №1, mix thoroughly.

6.3. Microtitration strips. Before opening keep the bag at room temperature (+18…25°C) for 30 minutes. Open the bag and place required number of strips on strip holder. Put remaining strips back in plastic bag and close tightly. Keep at +2...8°C until expiry date stated on the label.

6.4. Wash buffer. Prepare the necessary volume of Wash buffer by dilution of Buffer P 10-fold with distilled water. For example:

5 ml of Buffer P + 45 ml of distilled water. Mix thoroughly, avoiding foaming.

Keep firmly closed. Store at room temperature (+18...25°C) for no more than 5days. The rest of the Buffer P should be stored firmly closed at +2...8°C until expiry date.

6.5. Conjugate E is ready for use. Once opened, store at +2… 8°C for no more than 1 month.

6.6. TMB Substrate solution is ready to use. Once opened, store at +2… 8°C for no more than 1 month.

6.7. Stop reagent is ready to use. Once opened, store at +2… 8°C until expiry date.

7. ASSAY PROCEDURE

7.1. All the components and serum samples should be brought to room temperature and stirred thoroughly before the assay. Assay scheme is given on the last page.

7.2. Perform each assay in duplicate for both calibrators and unknowns.

7.4. Pipette 50 μl of the TSH calibrators, control and serum specimens into corresponding wells.

Note: total time of dispensing must not exceed 15 minutes, otherwise the test result may be unreliable, because the time of incubation with conjugate will substantially vary for different samples.

7.5. Pipette 100 μl of conjugate into each well **except A1 and A2.**

7.6. Incubate strips for 1 hour while shaking at 37°C.

7.7. decant, wash the strips five times with wash buffer. Each time add 300 μl of wash buffer per well and shake for 5–10 sec, then aspirate the buffer. After the last washing cycle, if

necessary, invert the plate and firmly tap on a clean paper towel to remove remaining wash buffer.

7.8. Add 100 µl of TMB Substrate solution into each well. Incubate at room temperature (+18…25^{o}C) in the dark for 15-30 minutes, depending on the color intensity.

7.9. Add 100 µl of Stop reagent to all wells and shake well for 1-2 minutes.

7.10. Read the optical density of the solution in the microwells at a wavelength of 450 nm.

8. PROCEDURAL NOTES

8.1. "ThyroidEIA-TSH" kit should be stored at + 2…8°C until expiry date stated on the label.

If used for separate experiments kit contents should be stored as follows:

- put remaining strips in plastic bag and close tightly. Keep at +2…8°C until expiry date;

- once opened, store Conjugate E and TMB substrate solution at + 2…8°C for no more than 1 month;

- once opened, store Buffer P at +2...8°C until expiry date;

- store prepared wash buffer firmly closed at room temperature for no more than 5 days;

- store calibrators and control at +2…+8°C for no more than 1month after opening;

- store Stop reagent at +2…8°C until expiry date.

8.2. Do not use plasma, hemolysed and lipemic serum or samples with sodium azide as a preservative.

8.3. Please take into consideration that calibrators should be measured in each separate assay. It is also recommended to measure TSH concentration in the control each time. The number of separate experiments you can perform with one kit (4 experiments) is therefore limited by volume of calibrators.

8.4. Do not use Stop reagents from other manufacturers.

8.5. The operator should thoroughly follow the manual to obtain the reliable result.

ATLAS MEDICAL
William James House, Cowley Road, Cambridge
Tel: ++44 (0) 1223 858 910
Fax: ++44 (0) 1223 858 524
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