Thyroid-stimulating hormone （TSH） ELISA Test Kit

NAME AND INTENDED USE

Detection Kit for thyroid-stimulating hormone（TSH）（Enzyme-Linked ImmunoSorbent Assay , ELISA）.It is used in quantitative tests for thyroid-stimulating hormone（TSH） in human serum.

SUMMARY AND CLINICAL SIGNIFICANCE

Thyroid-stimulating hormone (TSH) secretion is controlled by a negative feedback mechanism in which the variations of circulating thyroid hormone levels interact with the hypothalamic- pituitary axis to adjust TSH production.

TSH synthesis and secretion are also stimulated by TRH, a tripeptide produced by the hypothalamus and inhibited by somatostatin.

In the normal population, circulating TSH has been found, using this procedure, to be below 4.5 mIU/L under baseline conditions.

Investigation of a hyperthyroid population demonstrates an increased frequency of samples whose TSH levels are less than 0.1 mIU/L and at the detection limit. Development of sensitive determinations of TSH has therefore facilitated clinical interpretation of hypothalamic, pituitary and thyroid related desorders.

Indeed, elevated levels of T3 and T4 reduce TSH output by the pituitary. However, a single basal serum TSH determination is not considered to be discriminatory as such for the diagnosis of hyperthyroidism, which often requires a dynamic TRH test.

On the contrary, the demonstration of an elevated circulating TSH level is a clear indication for the development of primary hypothyroidism in most instances. In such situation, compl- ementary investigation of the pituitary/thyroid axis should be undertaken. Here again, the most useful dynamic test is the TRH stimulation test.

In case of simultaneous elevation of both TSH and thyroid hor- mone levels, the diagnosis should be oriented towards a state of thyroid hormone resistance and further documented by stimuli- tion(TRH) and inhibition(T3, T4)tests.

PRINCIPLE

TSH kit uses a “sandwich principle”, Enzyme-linked immunological sorbent assay. To measure TSH levels in serum, plastic wells coated with a monoclonal antibody of TSH are supplied in the kit. After the patient’s specimen and another mono-antibody labelled with HRP are added, TSH, if present, is fixed to the solid phase antibody and creating a HRP-antibody ~TSH~antibody “sandwich”. After TMB substrate added, the result is obtained by EIA plate reader.

PRECAUTION FOR USERS

1. Handling should preclude any pipetting by mouth.
2. Use only pipettes with disposable tips for each specimen.
3. Do not mix materials from different master lots. Do not use kit components beyond the expiration date. All materials should be brought to room temperature before use.

SPECIMEN COLLECTION AND PREPARATION

Serum specimens can be tested by the TSH procedure. Remove serum from the clot as soon as possible to avoid hemolysis. Covered specimens can be stored up to 48 hours at 2~8℃. Specimens held for a longer time can be frozen at –20℃ and avoid repeated freeze melting.

Serum samples with concentrations expected to be greater than 40 mIU/L should be diluted with normal saline.

NOTE: If needed, remove by centrifugation the suspended fibrin particles or aggregates which are liable to produce falsely positive results.

REAGENTS SUPPLIED

1. Coated Microplate: 1 plate (8×12 wells), Ready to use. Coated with anti-TSH antibody and sealed in an aluminum bag. Remove the strips in the resealable bag with a desiccant to protect from moisture after opened. Store at 2~8℃ until expiration date.
2. HRP Conjugate: 1 vial of 6ml, Ready to use. Store at 2~8℃ until expiration date.
3. Calibrator:,7 vials of 1ml, Ready to use. Labeled with S0 to S6 and the concentration of TSH is 0， 0.5, 1.5, 3.0, 6.0, 14.0,40.0mIU/L. Store at 2~8℃ until expiration date.
4. Control: 2 vials of 1ml, Ready to use. Store at 2~8℃ until expiration date. The concentration of low value is 1.61~2.99 mIU/L. The concentration of high value is 5.74~10.66 mIU/L. Store at 2~8℃ until expiration date.
5. Chromogen A: 1 vial of 7ml, Ready to use. Store at 2~8℃ until expiration date.
6. Chromogen B: 1 vial of 7ml, Ready to use. Store at 2~8℃ until expiration date.
7. Stop Solution: 1 vial of 7ml, Ready to use. Store at 2~8℃ until expiration date.
8. Wash buffer: 1 vial of 15ml, Concentrate 20-fold, diluting with deionized water before the assay. Store at 2~8℃ until expiration date.
9. Plate sealer: 2 pieces.
10. Plastic resealable bag: 1 set.
11. Instruction manual: 1 copy.

RELATED TIPS

1. This kit is designated for In-Vitro Diagnostic Use Only.
2. Wash procedure. Incomplete washing will adversely affect the test results. Wash each well 3 times with about 0.3ml wash buffer. If no automatic washer is available, washing can be performed manually as follows: Invert the plate vigorously to get all water out and block the rim of well on absorbent paper for a few seconds. Filling each well with water and remain 10 seconds. Repeat these steps 3 times. Blot dry the plate by inverting the plate onto absorbent tissue, and striking a hard surface several times.

3. Drip procedure. Mix the bottle gently before use. Violent surge may cause too much foam. Invert the bottle and squeeze one or two drop on absorbent tissue to make sure there is no foam. Take the bottle upright the well and make sure the drop does not touch the rim of wells.
4. Read procedure. Using the blank well to correct the zero point of reader if single wavelength reader is used. If double wavelength readers with 450nm and 630nm are used, there is no need to correct the zero point.
5. Storage. The whole kit should be stored at 2~8℃. Microplate should be taken to room temperature before opened. This is very important because absorbed atmospheric moisture by cold plates significantly reduces their shelf life. After removing the required number of strips, the plate should be put in the plastic resealable bag with desiccants to minimize exposure to damp air.
6. Control serum is prepared with human serum, which is tested negative of HBV, HCV and HIV. But it should still be considered as capable of transmitting viral diseases.

PREPARATIONS

1. Allow all specimens and reagents to reach room temperature and mix thoroughly by gentle inversion before use.
2. Prepare Wash buffer by diluting Wash Concentrate 20-fold with deionized water. The diluted wash solution is stable in room temperature for at least one week.

ASSAY PROCEDURE:

1. Mark the microtitration strips to be used. All the Calibrators and controls should set duplicate.
2. Dispense 100μl of Calibrators/controls/samples into wells.
3. Dispense 50μl of HRP Conjugate to each well.
4. Covered the strips with a plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 1 hours.
5. Wash each well for 3 times, 10 seconds each time. (See wash procedure).
6. Dispense 50μl of chromogen A to each well.
7. Dispense 50μl of chromogen B to each well.
8. Covered the strips with a fresh plate sealer. Mix it gently by swirling the microtiter plate on flat bench. Incubate the plate at 37°C for 15 minutes.
9. Dispense 50μl of stop solution to each well and mix completely.
10. Read the absorbance of the plate within 10 minutes. (See read procedure)

CALCULATION OF RESULTS

Computer: Use the linear fitting function, the logarithm of each calibrator concentration (Log), as X, take the logarithm of the corresponding absorbance value (Log(OD)) as Y, choose double logarithm (or full Logarithmic) Log-Log fitting the concentration of the serum to be tested is calculated from the fitted line.

Equation: log OD=B*log [concentration]+A

PERFORMANCE CHARACTERISTICS

1. Expected value

The cut-off value of TSH concentration for healthy subjects is 0.4~6.2 mIU/L.

2. Sensitivity

The detection limit of the assay is approximately 0.15 mIU/L.

3. Precision CV＜15%.
4. Specificity: No cross reactions with LH, FSH and HCG.

CODIGO: RSET102-3