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Product Information

Thyroid Stimulating Hormone (TSH) ELISA kit

Catalog Number: EA100840
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use
The TSH ELISA Kit is intended for the quantitative measurement of TSH in human serum.

Background
Thyroid Stimulating Hormone (TSH) is a glycoprotein hormone secreted by the pituitary gland and regulates the synthesis/ release of T3 and T4 by thyroid gland. TSH has two subunits, namely alpha and beta. The alpha subunit is similar to the alpha subunit found in LH, FSH and hCG glycoprotein hormones. However, the beta subunit is specific and differs from hormone to hormone. The serum TSH measurement is one of the most important tools in the diagnosis of thyroid disorders. Increased serum TSH is an early and sensitive indicator of decreased thyroid reserve and overt primary hypothyroidism. Decreased of TSH levels is an indicator of TSH-independent hyperthyroidism (Graves disease). The sensitivity of this ELISA test is 0.05 µIU/ml.

Principle of the test
The TSH is a solid phase sandwich ELISA method. The samples and anti-TSH- HRP/Biotin conjugate are added to the wells coated with Streptavidin. TSH in the patient's sample forms a sandwich between two specific antibodies to TSH. Unbound protein and HRP conjugate are washed off by wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of TSH in the samples. A standard curve is prepared relating color intensity to the concentration of the TSH.

Components

MATERIALS PROVIDED	96 Tests
1. Microwells coated with Streptavidin	12x8x1
2. TSH Standard: 7 vials (ready to use)	0.5ml
3. TSH Conjugate Reagent: 1 bottle (ready to use)	12 ml
4. TMB Substrate: 1 bottle (ready to use)	12ml
5. Stop Solution: 1 bottle (ready to use)	12ml
6. 20X Wash concentrate: 1 bottle	25ml

Materials and Equipment Required but Not Provided
1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm

5. Absorbance paper or paper towel
6. Graph paper
7. Microplate shaker

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection Handling

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

Reagent Preparation

Prepare 1X Wash buffer by adding the Wash concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (20-25°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (20-25°C).
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.
- Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipet 50 µl of TSH standards, control and patient specimens into designated wells.
3. Add 100 µl of ready to use conjugate reagent to all wells. Shake for (10-30) sec.
4. Cover the plate and incubate for 60 minutes at room temperature (20-25°C), with shaking (at 600 rpm).
5. Remove liquid from all wells. Wash wells four times with 300 µl of 1X wash buffer. Blot on absorbent paper towels.
6. Add 100 µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature (DO NOT Shake).
8. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

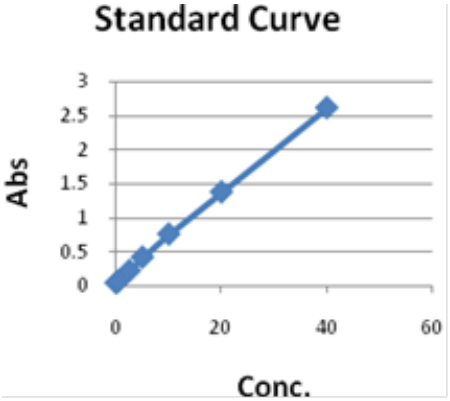
Calculation of Results

The standard curve is constructed as follows:

1. Check TSH standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the TSH standards (vertical axis) versus the TSH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Example of a Standard Curve

	OD 450 nm	Conc. µIU/ml
Std 1	0.033	0
Std 2	0.062	0.5
Std 3	0.21	2.5
Std 4	0.41	5
Std 5	0.75	10
Std 6	1.37	20
Std 7	2.61	40



References

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