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Product Information

Thyroid Stimulating Hormone (TSH) ELISA

Catalog Number **SE120135**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Thyroid Stimulating Hormone (TSH) is a glycoprotein hormone secreted by the pituitary gland and regulates the synthesis/release of T_3 and T_4 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.

The Thyroid Stimulating Hormone (TSH) ELISA is intended for the quantitative measurement of TSH in human serum. It 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 sample forms a sandwich between two specific antibodies to TSH. Unbound protein and HRP conjugate are washed off. 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
Microwells coated with Streptavidin	12 x 8 x 1
TSH Standard: 7 vials (ready to use)	0.5 mL
TSH Conjugate Reagent: 1 bottle (ready to use)	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
20x Wash concentrate: 1 bottle	25 mL

Reagents 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 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

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.

20x Wash Buffer Concentrate

Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water. Store at room temperature (18–26 °C).

Storage/Stability

Store the kit at 2–8 °C.

Procedure

Notes: 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.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Prior to assay, allow reagents to stand at room temperature (18–26 °C). Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder
2. Pipette 50 µL of TSH standards, control, and specimens into designated wells.
3. Add 100 µL of ready to use conjugate reagent to all wells. Shake for 10–30 seconds.
4. Cover the plate and incubate for 60 minutes at room temperature (18–26 °C).
5. Remove liquid from all wells. Wash wells three 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.
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.

Results

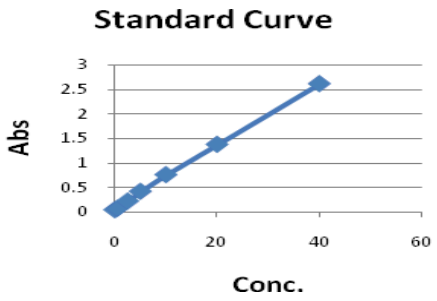
Calculations

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 the value is checked on every kit.
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 Standard Curve

	OD (450 nm)	Conconcentration 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



Expected values

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for TSH may be used as initial guideline ranges only:

Classification	Normal Range (µIU/mL)
Adults	0.4–4.2
Newborn (1–4 days)	1.0–39
2–20 weeks	1.7–9.0
21 weeks – 20 years	0.7–6.4

References

1. Frank, J.E. et al., Thyroid function in very low birth weight infants: effects on neonatal hypothyroidism screening. J. Pediatr., 1996;128(4):548–54.
2. Thakur, C. et al., Total serum levels of triiodothyronine (T3) thyroxine (T4) and thyrotropine (TSH) in school going children of Dibrugarh district: an endemic goitre region of Assam. Indian J. Physiol. Pharmacol., 1997;41(2):167–70.
3. Morimoto, K., and Inouye, K.A., Sensitive enzyme immunoassay of human thyroid–stimulating hormone (TSH) using bispecific F(ab')2 fragments recognizing polymerized alkaline phosphatase and TSH. J. Immunol. Methods, 1997;205(1):81–90.
4. Maes, M. et al., Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clin. Endocrinol. (Oxf), 1997; 46(5):587–98

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