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Thyroid Stimulating Hormone (human) ELISA Kit

rev 02/17

(Catalog # K7411-100, 100 assays; Store at 2-8^{0}C)

I. Introduction:

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. Increased serum TSH is an early and sensitive indicator of decreased thyroid reserve and overt primary hypothyroidism. Decrease TSH levels is an indicator of TSH-independent hyperthyroidism (Graves disease). BioVision's human TSH kit is a solid phase sandwich ELISA Kit. The samples, and anti-TSH-HRP conjugate are added to the wells coated with monoclonal antibody to TSH beta subunit. TSH in the sample binds to anti-TSH MAb on the well and the anti-TSH detection antibody then binds 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. The sensitivity of this ELISA test is 0.5 µIU/ml.

II. Application:

Quantitative protein detection, establishing normal range etc.

III. Specificity:

Human TSH.

IV. Sample Type:

- Serum

V. Kit Contents:

Components	K7411-100	Part No.
Microplate coated with TSH MAb, 96 wells	12 stripsx8 wells	K7411-100-1
TSH Standard: (0.5 ml) (ready to use)	7 vials	K7411-100-2
TSH Enzyme Conjugate (ready to use)	12 ml	K7411-100-3
Wash Concentrate (20X)	25 ml	K7411-100-4
TMB Substrate (ready to use)	12 ml	K7411-100-5
Stop Solution (ready to use)	12 ml	K7411-100-6

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips.

VII. Storage Conditions and Reagent Preparation:

Store kit at 2-8^{0}C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

- **Wash 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).

VIII. Warning & Precautions:

- Potential biohazardous materials: The standards set contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Do not pipette by mouth.
- 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.
- 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.

IX. Sample Preparation and Storage:

Collect blood specimens and separate the serum immediately. 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. Avoid multiple freeze-thaw cycles. Prior to assay, frozen sera should be completely thawed and mixed well. Do not use grossly lipemic specimens. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

X. Assay Protocol:

Prior to assay, allow reagents to stand at room temperature. Gently mix all reagents before use. 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.

1. Place the desired number of coated strips into the holder
2. Pipet 50 µl of TSH standards, control, and samples into designated wells.
3. Add 100 µl of TSH enzyme conjugate to all wells. Shake for 10-30 sec.
4. Cover the plate and incubate for 60 min. 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 & incubate for 15 min. at room temperature.
7. Add 50 µl of stop solution to all wells. Shake the plate gently to mix the solution.
8. Read absorbance on ELISA Reader at 450 nm within 15 min. after adding the stopping solution.

XI. Calculation: Construct the standard curve; plot the absorbance for the TSH standards (vertical axis) versus the TSH standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample. Value above the highest point of the standard are retested after diluting with "0" standard.

Example of a Standard Curve:

Standard	OD (450 nm)
Standard 1 (0 ng/ml)	0.033
Standard 2 (0.5 ng/ml)	0.062
Standard 3 (2.5 ng/ml)	0.21
Standard 4 (5 ng/ml)	0.41
Standard 5 (10 ng/ml)	0.75
Standard 6 (20 ng/ml)	1.37
Standard 7 (40 ng/ml)	2.61

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

XII. RELATED PRODUCTS:

Human CellExp™ TPO, human recombinant (6483) TPO (mouse) ELISA Kit (4753)

FOR RESEARCH USE ONLY! Not to be used on humans.