Human TSH ELISA Kit

Enzyme Immunoassay for the quantitative determination of human thyrotropin (TSH) in serum and plasma

Catalog number: ARG80894

Package: 96 wells

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MANUFACTURED BY:

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INTRODUCTION

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 daltons and secreted from the anterior pituitary, is generally regarded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism . Increase in serum concentrations of TSH, which is primarily responsible for the synthesis and release of thyroid hormones, is an early and sensitive indicator of decrease thyroid reserve and in conjunction with decreased thyroxine (T4) concentrations is diagnostic of primary hypothyroidism. The expected increase in TSH concentrations demonstrates the classical negative feedback system between the pituitary and thyroid glands. That is, primary thyroid gland failure reduces secretion of the thyroid hormones, which in turn stimulates the release of TSH from the pituitary. Additionally, TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduces the response of the pituitary to the stimulatory effects of TRH. In secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH levels are generally low or normal. Either pituitary TSH deficiency (secondary hypothyroidism) or insufficiency of stimulation of the pituitary by TRH (tertiary hypothyroidism) causes this. The TRH stimulation test differentiates these conditions. In secondary hypothyroidism, TSH response to TRH is blunted while a normal or delayed response is obtained in tertiary hypothyroidism. Further, the advent of

immunoenzymometric assays has provided the laboratory with sufficient sensitivity to enable the differentiating of hyperthyroidism from euthyroid population and extending the usefulness of TSH measurements. This method is a second-generation assay, which provide the means for discrimination in the hyperthyroid-euthyroid range.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for TSH has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any TSH present is bound by the immobilized antibody. After washing away any unbound substances, HRP-TSH antibody conjugate is added and incubate. Then washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of TSH bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of TSH in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C
HRP-antibody Conjugate	12 ml (ready to use)	4°C
Standards 0-5 (0, 0.25, 0.75, 2.0, 5.0, 15 mIU/L)	6 X 0.4 ml (ready to use)	4°C
Control Low (2.48 –4.14 mIU/L)	0.4 ml (ready to use)	4°C
Control High(5.49 –9.15 mIU/L)	0.4 ml (ready to use)	4°C
40X Wash buffer	25 ml	4°C
Substrate (TMB)	12 ml (ready to use)	4°C
Stop solution	14 ml (ready to use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
- Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above.
- Briefly spin down the HRP-antibody conjugate before use.
- If crystals are observed in the 40X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the assay has been started, all steps should be completed without interruption.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 40X wash buffer with distilled water to yield 1X wash buffer. E.g.: Add 25 ml of 40X Wash buffer into 975 ml of distilled water to a final volume of 1000 ml, mix thoroughly. The diluted Wash buffer is stable for 2 weeks at RT.
- Sample: If the initial assay found samples contain TSH higher than the highest standard, the samples can be diluted with Standard 0 and re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  Example:

  a) Dilution 1:10: 10 µl sample + 90 µl Standard 0 (mix thoroughly).

  b) Dilution 1:100: 10 µl 1:10 diluted a) + 90 µl Standard 0 (mix thoroughly).

  Note: it is recommended to do pre-test to determine the suitable dilution factor.

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT; 21-26 °C) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **25 µl standards, controls** and **samples** into each well. Incubate for **10 minutes** at **RT**.
3. Add **100 µl HRP-antibody conjugate** into each well. Mix thoroughly for 10 sec.
4. Cover wells and incubate for **90 minutes** at **RT**.
5. Aspirate each well and **wash**, repeating the process 4 times for a **total 5 washes**. Wash by filling each well with wash buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining distilled water by aspirating, decanting or blotting against clean paper towels.
6. Add 1**00 µl** of **TMB Substrate** to each well. Incubate for **20 minutes** at **room temperature in dark**.
7. Add **100 µl Stop solution** into each well.
8. Read the OD with a microplate reader at **450 nm** immediately. It is recommended that the wells be read within 5 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi logarithmic or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 15 mIU/L. For the calculation of the concentrations this dilution factor has to be taken into account.

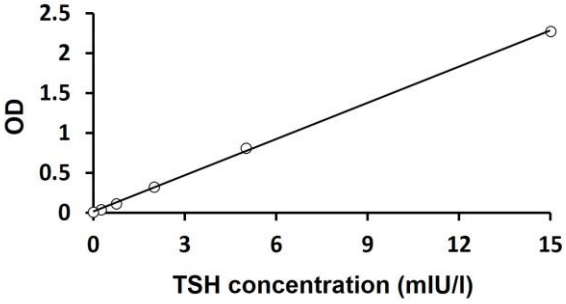
5. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

6. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

7. The diluted samples must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimal detectable concentration is approximately 0.06 mIU/L.

Specificity

The cross-reactivity of the TSH ELISA method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations.

No cross-reactions were found when testing up to

100,000 mIU/mL Chorionic Gonadotropin (hCG)

100 mIU/mL Follicle Stimulating Hormone (hFSH)

100 mIU/mL Luteinizing Hormone (hLH)

Intra-assay and inter-assay precision

The CV value of intra-assay precision was 3.47% and the CV value of inter-assay precision was 5.94%.

Interfering Substances

Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.

Drug Interferences

Serum thyrotropin values may be elevated by pharmacological intervention. Domperiodone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.

Limitations of use

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice.

Any improper handing of sample or modification of this test might influence the result.