Human TSH ELISA Kit

For the quantitative determination of human thyroid stimulating hormone (TSH) concentrations in serum.

Catalogue Number: EL10012

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (03) TSH

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INTENDED USE

This Human TSH ELISA Kit is to be used for the *in vitro* quantitative determination of human thyroid stimulating hormone (TSH) concentrations in serum. This kit is intended LABORATORY FOR RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

The determination of serum or plasma levels of thyroid-stimulating hormone (TSH, or thyrotropin) is recognized as a sensitive method in the diagnosis of primary and secondary hypothyroidism. Thyroid-stimulating hormone is secreted by the anterior lobe of the pituitary gland and induces the production and release of thyroxine and triiodothyronine from the thyroid gland. It is glycoprotein with a molecular weight of approximately 28,000 Daltons, consisting of two chemically different subunits, alpha and beta.

Although the concentration of TSH in the blood is extremely low, it is essential for the maintenance of normal thyroid function. The release of TSH is regulated by a TSH-releasing hormone (TRH) produced by the hypothalamus. The levels of TSH and TRH are inversely related to the level of thyroid hormone. When there is a high level of thyroid hormone in the blood, less TRH is released by the hypothalamus, so the pituitary secretes less TSH. The opposite action will occur when there is decreased thyroid hormone in the blood. This process is known as a negative feedback mechanism and is responsible for maintaining the proper blood levels of these hormones.

TSH and the pituitary glycoproteins: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG) have identical alpha chains. The beta chain is distinct but does contain identical amino acid sequences, which can cause considerable cross-reactivity with some polyclonal TSH antisera. The use of monoclonal antibodies in this Human TSH ELISA Kit eliminates this interference, which could result in falsely elevated TSH values in either menopausal or pregnant females, a population whose evaluation of thyroid status is clinically significant.

PRINCIPLE OF THE ASSAY

This TSH enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for TSH. Standards or samples are then added to the microtiter plate wells and TSH if present, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of TSH present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated monoclonal antibody, specific for TSH are added to each well to “sandwich” the TSH immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Next, a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain TSH and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.

In order to measure the concentration of TSH in the sample, this Human TSH ELISA Kit includes a set of calibration standards (6 standards). The calibration standards are assayed at

the same time as the samples and allow the operator to produce a standard curve of Optical Density (O.D.) versus TSH concentration (μIU/mL). The concentration of TSH in the samples is then determined by comparing the O.D. of the samples to the standard curve.

REAGENTS PROVIDED

All reagents provided are stored at 2-8° C. Refer to the expiration date on the label.

	96 tests

1. **MICROTITER PLATE** (Part EL12-1) ***96 wells***
   Pre-coated with anti-human TSH monoclonal antibody.
2. **CONJUGATE** (Part EL12-2) ***12 mL***
   Anti-human TSH monoclonal antibody conjugated to horseradish peroxidase (HRP) with preservative. *Ready-to-use.*
3. **STANDARD - 40 μIU/mL** (Part EL12-3) ***1 vial***
   Lyophilized human TSH in a buffered protein base with preservative that will contain 40 μIU/mL after reconstitution.
4. **STANDARD - 20 μIU/mL** (Part EL12-4) ***1 vial***
   Lyophilized human TSH in a buffered protein base with preservative that will contain 20 μIU/mL after reconstitution.
5. **STANDARD - 10 μIU/mL** (Part EL12-5) ***1 vial***
   Lyophilized human TSH in a buffered protein base with preservative that will contain 10 μIU/mL after reconstitution.
6. **STANDARD - 5 μIU/mL** (Part EL12-6) ***1 vial***
   Lyophilized human TSH in a buffered protein base with preservative that will contain 5 μIU/mL after reconstitution.
7. **STANDARD - 1 μIU/mL** (Part EL12-7) ***1 vial***
   Lyophilized human TSH in a buffered protein base with preservative that will contain1 μIU/mL after reconstitution.
8. **STANDARD - 0 μIU/mL** (Part EL12-8) ***1 vial***
   Lyophilized buffered protein base with preservative that will contain 0 μIU/mL of human TSH after reconstitution.
9. **SUBSTRATE A** (Part EL12-9) ***10 mL***
   Buffered solution with H_20_2.
10. **SUBSTRATE B** (Part 30007) ***10 mL***
    Buffered solution with TMB.
11. **STOP SOLUTION** (Part 30008) ***14 mL***
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100 µL and 50-200 µL for running the assay.
2. Pipettes: 1 mL, 5 mL, and 10 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Incubator (37±2°C)
7. Microtiter plate reader (450 nm±2 nm)
8. Automatic microtiter plate washer or squirt bottle
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Waste: Autoclave 60 min. at 121°C.
   Liquid Waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.

SAMPLE PREPARATION

COLLECTION, HANDLING AND STORAGE

Serum: Blood should be drawn using standard venipuncture techniques and serum separated from blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C) and serum extracted. This kit is for use with serum samples without additives only.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum samples to be used within 24-48 hours may be stored at 2-8°C otherwise samples must be stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below.

1. **TSH Standards:** Reconstitute each TSH Standard vial with **0.6 mL** of deionized or distilled water. Allow each solution to sit for at least 15 minutes with gentle agitation. The TSH standard stock solutions are stable at 4°C for 3 months. Avoid freeze-thaw cycles

2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table below for correct amounts of Substrate Solution to prepare.

Wells Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
16 wells	1.5	1.5	3.0
32 wells	3.0	3.0	6.0
48 wells	4.0	4.0	8.0
64 wells	5.0	5.0	10.0
80-wells	6.0	6.0	12.0
96 wells	7.0	7.0	14.0

ASSAY PROCEDURE

1. Prepare all TSH Standards before starting assay procedure (see Preparation Reagents). *It is recommended that all Standards and Samples be added in duplicate to the Microtiter Plate.*

2. First, secure the desired number of coated wells in the holder, then add 50 µL of Standards or Samples to the appropriate well of the antibody pre-coated Microtiter Plate.

3. Add 2 drops or 100 µL of Conjugate to each well. Mix well. ***Complete mixing in this step is important.*** Cover and incubate for 2 hours at 37°C.

4. Prepare Substrate Solution no more than 15 minutes before end of incubation (see Preparation of Reagents).

5. Wash the Microtiter Plate using one of the specified methods indicated below:

   Manual Washing: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with distilled or de-ionized water, then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.

   Automated Washing: Aspirate all wells, then wash plate **FIVE times** using distilled or de-ionized water. Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

6. Add 100 µL Substrate Solution to each well. Cover and incubate for 15 minutes at 37°C.

7. Add 100 µL of Stop Solution to each well. Mix well.

8. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 30 minutes.

CALCULATION OF RESULTS

This standard curve is used to determine the amount of thyroid stimulating hormone (TSH) in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the six standard concentrations on the vertical (Y) axis versus the corresponding TSH concentration (µIU/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the zero standard (0 μIU/mL) before result interpretation. Construct the standard curve using graph paper or statistical software.
2. To determine the amount of TSH in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding TSH concentration.

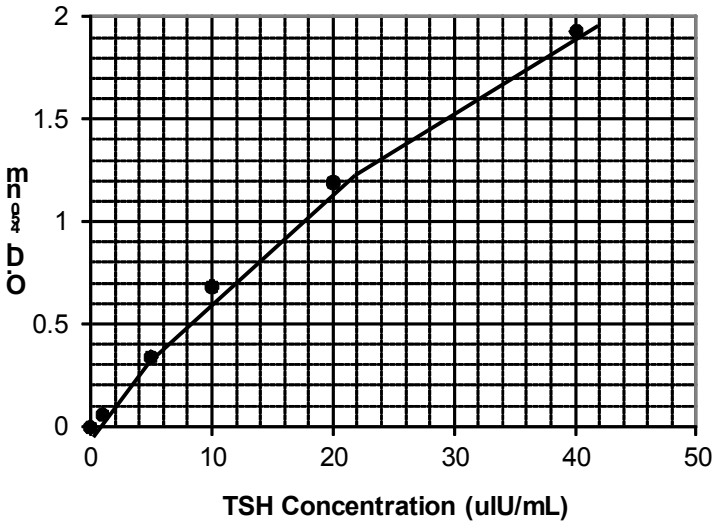
TYPICAL DATA

Results of a typical standard run of TSH ELISA are shown. Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain their own standard curve.

EXAMPLE

Results of a typical standard run are shown below:

Standard (μIU/mL)	O.D. (450 nm)	Mean	Zero Standard Subtracted
0	0.024, 0.021	0.023	0
1	0.078, 0.088	0.083	0.060
5	0.343, 0.387	0.365	0.342
10	0.687, 0.729	0.708	0.685
20	1.233, 1.188	1.211	1.188
40	1.941, 1.951	1.946	1.923



PERFORMANCE CHARACTERISTICS

1. **SENSITIVITY**
   The minimal detectable concentration of TSH by this assay is estimated to be 0.2 μIU/mL.

2. **SPECIFICITY**
   This kit exhibits no detectable cross-reaction with human FSH, LH, Prolactin, or CG. Human GH can be detected in this assay.

3. **CALIBRATION**
   This immunoassay is calibrated against WHO, 2nd IRP, 80/558.

4. **HOOK EFFECT**
   In this assay, no hook effect is observed up to 2000 μIU/ml.

5. **EXPECTED NORMAL VALUES**
   Each laboratory must establish its own normal ranges based on patient population. The results provided below are based on 160 random normal adult blood specimens.

Sample (n)	**Mean TSH** (μIU/ml)	**Range** (μIU/ml)
160	1.6	0.4 – 7.0