TRUSTwell®

Thyroid Stimulating Hormone (TSH) ELISA Kit

IVD REF **AE1030**

- 96-well ELISA kit for the quantitative determination of TSH concentration in human serum
- Store at 2-8°C upon receipt

INTENDED USE

The TSH ELISA Kit is a solid-phase enzyme-linked immunosorbent assay for the quantitative determination of Thyroid Stimulating Hormone (TSH) concentration in human serum. It is intended to be used by professionals as an aid in the diagnosis of thyroid dysfunction.

INTRODUCTION

Human thyroid stimulating hormone (TSH) is synthesized by the basophilic cells (thyrotropes) of the anterior pituitary[1]. It is composed of two non-covalently linked subunits designated α and ß. The structure of the α subunit is similar to that of luteinizing hormone (LH), follicle stimulating hormone (FSH) and human chorionic gonadotropin (hCG). The ß subunit is hormone specific and confers biological as well as immunological specificity[2]. Both the α and ß subunits are required for its biological activity. TSH stimulates the production and secretion of the metabolically active thyroid hormones, thyroxine (T4) and triiodothyronine (T3). T4 and T3 are responsible for regulating diverse biochemical processes throughout the body which are essential for normal development as well as metabolic and neural activity[3].

The hypothalamic-pituitary-thyroid (HPT) axis is established by communication and feedback between the hypothalamus, the pituitary gland, and the thyroid. The synthesis and secretion of TSH from the anterior pituitary is stimulated by the hypothalamic tripeptide thyrotropin releasing hormone (TRH), which is released from the hypothalamus in response to low levels of circulating T4/T3. When the hypothalamus senses that T4/T3 hormones have resumed to normal levels, TRH and TSH release are inhibited. The hypothalamus may also inhibit TSH release via the exertion of somatostatin and dopamine. Failure at any level of regulation of the HPT axis will result in either underproduction (hypothyroidism) or overproduction (hyperthyroidism) of T4 and/or T3.

Recent data from large population studies have shown that the mean TSH level in the general population is approximately 1.50 µIU/mL (95% confidence interval 1.46-1.54 µIU/mL)[4,5]. In the United States, hypothyroidism occurs in about 4.6% of the adult population, and hyperthyroidism is present in 1.3% of population[6]. Most physicians consider TSH levels > 5-10 µIU/mL as evidence of mild or subclinical hypothyroidism, and TSH levels < 0.2-0.4 µIU/mL as evidence of hyperthyroidism[7]. However, it is important for each facility to establish its own TSH reference interval based on representative sampling of the local population.

TEST PRINCIPLE

The TSH ELISA Kit is a solid-phase enzyme-linked immunosorbent assay based on the principle of antibody sandwich technique for the quantitative determination of TSH concentration in human serum.

The TSH ELISA Kit is composed of two key components:

1) Solid microwells pre-coated with monoclonal anti-TSH antibody, specific for the β-subunit.
2) Liquid conjugate composed of monoclonal anti-intact TSH antibody conjugated with horse radish peroxidase (HRP-anti-TSH Conjugate).

During the assay, the test specimen and HRP-anti-TSH Conjugate, are incubated simultaneously with the coated microwells. The TSH, if present in the specimen, binds to both the anti- β TSH antibody on the microwell surface and the anti-TSH antibody in the HRP Conjugate, forming an antibody sandwich immunocomplex.

Unbound conjugate is then removed by washing. The presence of the conjugate complex is shown by the development of a blue color upon an additional incubation with the substrate. The reaction is terminated with Stop Solution, and the absorbance determined using a spectrophotometer at 450/620-690 nm. A standard curve is generated by plotting the absorbance at 450/620-690 nm wavelength versus the respective TSH concentration for each standard. The concentration of TSH in the samples is then determined directly from this curve.

MATERIALS AND REAGENTS

Materials and reagents provided with the kit

Item	Description	Quantity	Catalog
1	Anti-TSH Ab Coated Microwells	8 wells x 12 strips	AE1030W
2	TSH Standards: S1 (0 µIU/mL)	1 mL	AE1030S1
3	S2 (0.5 µIU/mL)	0.75 mL	AE1030S2
4	S3 (2 µIU/mL)	0.75 mL	AE1030S3
5	S4 (5 µIU/mL)	0.75 mL	AE1030S4
6	S5 (10 µIU/mL)	0.75 mL	AE1030S5
7	S6 (20 µIU/mL)	0.75 mL	AE1030S6
8	S7 (40 µIU/mL)	0.75 mL	AE1030S7
9	HRP-anti-TSH Conjugate	6 mL	AE1030H
10	TMB Substrate	12 mL	ATME2000
11	Wash Buffer Concentrate (40X)	20 mL	AWE3002
12	Stop Solution	12 mL	ASE1000
13	Product Insert	1	PI-AE1030
14	ELISA Working Sheet	2	AE0001ES
15	Microplate Sealers	3	
16	Desiccant	4	

Materials and reagents required but not provided in the kit

1. Pipette capable of delivering 50 µL, 100 µL, 200 µL, and 1 mL
2. Microplate reader with a bandwidth of 10 nm or less and an optical density range of 0-2 OD or greater at 450/620-690 nm wavelength is acceptable
3. Vortex mixer or equivalent
4. Absorbent paper for blotting the microwells
5. Graph paper
6. Timer
7. Distilled or de-ionized water

STORAGE AND STABILITY

All reagents except the concentrated wash buffer are ready to use as supplied. Store all components at 2-8°C. Do not freeze. Avoid strong light. Reseal the microwells after removing the desired number of wells. Ensure that the reagents are brought to room temperature before opening. All the reagents are stable through the expiration date printed on the label if not opened. Place unused wells in the resealable bag with desiccant and return to 2-8°C.

SPECIMEN COLLECTION AND PREPARATION

- Serum should be prepared from a whole blood specimen obtained by acceptable venipuncture technique.
- This kit is designed for use with serum specimen without additives only.
- If a specimen is not tested immediately, refrigerate at 2-8°C. If a storage period greater than three days is anticipated, the specimen should be frozen (-20°C). Avoid repeated freeze-thawing of specimens. If a specimen is to be shipped, pack in compliance with federal regulations covering the transportation of etiologic agents.
- Specimens containing precipitants may give inconsistent test results. Clarify such specimens by centrifugation prior to performing the assay.
- Do not use serum specimens demonstrating gross lipemia, gross hemolysis or turbidity. Do not use specimens containing sodium azide.

PREPARATION OF THE REAGENTS PRIOR TO ASSAYING

1. Bring all reagents, controls to room temperature (20-25°C).
2. **Preparation of working Wash Buffer:**
   If precipitants are visible, warm up the Wash Buffer (40X concentrate) at 37°C. Dilute concentrated Wash Buffer 40-fold with water as follows:

Plate	DI water	40 X wash buffer	Final volume
Full plate	195 mL	5 mL	200 mL
Half plate	97.5 mL	2.5 mL	100 mL
Quarter plate	48.75 mL	1.25 mL	50 mL

3. Mix each reagent before adding to the test wells.
4. Determine the number of strips needed and mark on the ELISA working sheet with the appropriate information. Standards should be run in duplicate to ensure accuracy.

   Dilute samples with expected TSH concentration over 40 µIU/mL with Standard S1. Mix diluted samples thoroughly prior to performing the assay.

ASSAY PROCEDURE

1. Remove the desired number of strips and secure them in the microplate frame. Place unused strips into the resealable bag along with desiccant and seal for later use.
2. Add 50 µL of the TSH Standards, controls if applicable, and patient specimen into the assigned wells.
3. Dispense 50 µL of HRP-anti-TSH Conjugate into all the wells.
4. Shake the microplate gently for 30 seconds, then cover the plate with a microplate sealer.
5. Incubate the wells at 37ºC for 60 minutes.
6. Wash Step (Can be performed manually or with automated washing):
   Manual washing: Carefully remove the incubation mixture by disposing the solution into a waste container. Fill each well with 350 µL working wash buffer and rock gently for 20-30 seconds. Discard the wash solution completely. Repeat 4 more times. After completing the last wash step, tap the plate on absorbent paper to remove residual liquid.

   Automated washing: Automatic plate washer must be calibrated to ensure efficient washing. Fill each well with 350 µL diluted wash buffer and soak for 20-30 seconds. Aspirate all wells completely. Repeat 4 more times.
7. Add 100 µL of TMB Substrate into each well, and gently mix.
8. Incubate at room temperature (20-25ºC) in the dark for 20 minutes.
9. Stop the reaction by adding 100 µL of Stop Solution to each well. Gently mix for 30 seconds. **It is important to ensure that all the blue color completely changes to a color yellow.**
10. Set the microplate reader wavelength at 450 nm and measure the absorbance (OD) of each well within 15 minutes after adding Stop Solution. A filter of 620-690 nm can be used as a reference wavelength to optimize the assay result.

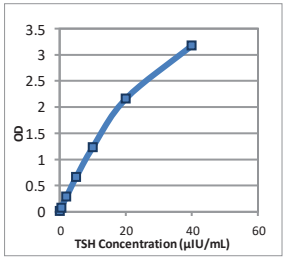
CALCULATION OF RESULTS

1. Calculate the mean absorbance value ($A_{450/620-690}$) for each set of standards.
2. Construct a standard curve by plotting the mean value (or subtracted mean value) obtained for each standard against its concentration on graph paper with absorbance values on the vertical Y axis, and concentrations on the horizontal X axis.
3. Use the absorbance values (or subtracted absorbance values) for each specimen to determine the corresponding concentration of TSH in µIU/mL from the standard curve.
   Alternatively, if software is used, calculate the concentration of TSH following software menu.
4. Any values obtained for a diluted sample must be further converted by applying the appropriate dilution factor in the calculation.

INTERPRETATION OF RESULTS

1. Results of a typical standard curve are shown below:

TSH (µIU/mL)	OD A450/620-690
0.0	0.012
0.5	0.077
2.0	0.286
5.0	0.665
10	1.233
20	2.163
40	3.175



EXPECTED NORMAL VALUE

1. 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 can be used as initial guideline ranges only:

Classification	Number	Mean TSH (µIU/mL)	Range[5]
Normal	231	1.50	0.45-4.12
Hypothyroid	16	11.20	> 5.0
Hyperthyroid	3	0.12	< 0.2

PERFORMANCE CHARACTERISTICS

1. Analytical Sensitivity

OD values from 20 replicates of the '0' calibrator was run in a single assay on three lots of the TSH ELISA Kit and the mean and standard deviation (SD) were calculated. The analytical sensitivity was determined to be 0.035 µIU/mL at 2SD.

2. Analytical Specificity

Specificity was determined by spiking concentrated high concentration of hCG, FSH, and LH into two pooled human serum samples. Each sample was compared to the un-spiked control. The results showed that no cross-reactivity with hCG, FSH, and LH was detected at the concentrations tested.

Substance	Cross-reactivity Ratio	Concentration
hTSH	1.00	-
hCG	$<1x10^{-9}$	200,000 mIU/mL
FSH	$<1x10^{-6}$	500 mIU/mL
LH	$<1x10^{-6}$	500 mIU/mL

3. Accuracy

In a total of 156 specimens, TSH levels were measured by the TSH ELISA Kit and a reference CLIA. Results are presented as average of 3 lot's performance at 95% confidence level.

Regression Analysis		Correlation Coefficient
Slope	Intercept	
0.991 ± 0.09	0.122 ± 0.122	0.986 ± 0.004

4. Precision

a. Intra-assay precision was determined by assaying 20 replicates of three patient pools. The mean, SD, and coefficient of variation (CV) (%) are shown in the following table:

Panel	N	Mean (µIU/mL)	SD	%CV
Low	20	0.597	0.034	5.8
Middle	20	4.817	0.418	8.7
High	20	19.747	1.538	7.8

b. Inter-assay precision was determined by assaying three patient pools in 10 separate runs over 3 lots. The mean, SD and CV (%) are shown in the following table:

Panel	Runs	Mean (µIU/mL)	SD	%CV
Low	10	0.397	0.035	8.9
Middle	10	4.800	0.367	7.6
High	10	31.118	2.005	6.4

5. Hook effect

No hook effect was observed at the TSH concentration up to 8,000 µIU/mL.

6. Interference

Common substances (such as pain, fever medication, and blood components) may affect the performance of the TSH ELISA Kit. This was studied by spiking these substances into two independently qualified human serum pools at normal and elevated levels of TSH, respectively. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the TSH ELISA Kit. The list of potentially interfering substances and concentrations tested are shown in the following table:

1. Acetaminophen	1324 uM	6. Diltiazem	15 uM
2. Erythromycin	81.6 uM	7. Hemoglobin	2 g/dL
3. Verapamil	4.4 uM	8. Bilirubin	20 mg/dL
4. T3	100 ng/mL	9. HAMA	52.5 ng/mL
5. T4	100 ug/dL	10. RF	215 IU/mL

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to check on assay performance. Control containing sodium azide cannot be used. Any material used should be assayed repeatedly to establish mean values and acceptable ranges to assure proper performance. This kit does not include serum controls.

WARNING AND PRECAUTIONS

1. This package insert must be read completely before performing the test. Failure to follow the insert gives inaccurate test results.
2. Do not use expired kits.
3. Bring all reagents to room temperature (20-25°C) before use.
4. Do not use the components of any other type of test kit as a substitute for the components in this kit.
5. Do not use serum derived from hemolyzed blood specimens for testing.
6. Do not ingest the reagents. Avoid contact with eyes, skin and mouth. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
7. Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
8. Users of this test should follow the US CDC Universal Precautions for prevention of transmission of HIV, HBV and other blood-borne pathogens.
9. Dispose of all specimens and materials used to perform the test as bio-hazardous waste.
10. At the beginning of each incubation and after adding Stop Solution, gently shake the microwells to ensure thorough mixing. Avoid the formation of air bubbles which results in inaccurate absorbance values. Avoid splashing liquid while rocking or shaking the wells.
11. Don't allow the microwells to dry between the end of the washing operation and the reagent distribution.
12. The enzyme reaction is very sensitive to metal ions. Thus, do not allow any metal element to come into contact with the conjugate or TMB Substrate.
13. The TMB substrate must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The TMB Substrate must be stored in the dark.
14. Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and TMB Substrate.
15. The wash procedure is critical. Wells must be aspirated completely before adding the Wash Buffer or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance values.
16. Avoid exposure to strong light during color development.

LIMITATIONS OF TEST

1. The Assay Procedure and the Interpretation of Results must be followed closely when assaying the levels of TSH in serum specimens from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The TSH ELISA kit is limited to the quantitative detection of TSH in serum.
3. The TSH assay cannot be used to detect pituitary and/or hypothalamic disease, as it may not be able to distinguish between normal and abnormal TSH isoforms that are present in these diseases.
4. Other non-thyroid disease can also change thyroid hormone metabolism and hypothalamic/pituitary function, resulting in decreased or increased TSH levels.
5. Accurate diagnosis of thyroid function relies on the determination of thyroid-stimulating hormone (TSH), free thyroxine (fT4), free Triiodothyronine (fT3), anti-TSH receptor autoantibody and assessment of patient's clinical history. A TSH assay alone cannot be used to determine the patient's clinical outcome.
6. Any interpretation or use of this test result must also rely on other clinical findings as well as on the professional judgment of health care providers.

REFERENCES

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