TSH ELISA Kit

CAT NO: SE101-01

In vitro Diagnostics

INTENDED USE

The Sensit TSH ELISA Kit is a solid phase enzyme linked immunosorbent assay for the qualitative detection of TSH in human serum or plasma. TSH is a useful marker for the diagnosis of hypothyroidism and hyperthyroidism. Any reactive specimen with the Sensit TSH ELISA Kit must be confirmed with alternative testing method(s) and clinical findings.

SUMMARY

Thyroid-stimulating hormone (TSH) is a glycoprotein hormone produced by the anterior pituitary gland. It plays a crucial role in regulating thyroid function by stimulating the thyroid gland to produce and release thyroid hormones—thyroxine (T4) and triiodothyronine (T3). These hormones are essential for metabolism, growth, and energy balance. TSH secretion is controlled by the hypothalamic-pituitary-thyroid (HPT) axis. The hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates the pituitary to secrete TSH. In turn, TSH stimulates the thyroid gland, and thyroid hormones exert negative feedback on both the pituitary and hypothalamus to regulate TSH levels. Abnormal TSH levels indicate thyroid dysfunction. Elevated TSH suggests hypothyroidism (underactive thyroid), often due to Hashimoto's thyroiditis or iodine deficiency. Low TSH levels suggest hyperthyroidism (overactive thyroid), commonly caused by Graves' disease or thyroid nodules. TSH measurement is the primary screening test for thyroid disorders. It is particularly useful in diagnosing subclinical hypothyroidism or hyperthyroidism before symptoms appear. Regular screening is recommended for individuals at risk, including pregnant women, the elderly, and those with a family history of thyroid disease.

TEST PRINCIPLE

Sensit TSH ELISA Kit is a solid phase enzyme linked immunosorbent assay based on the principle of antibody sandwich technique for the detection of TSH in human serum or plasma.

The Sensit TSH ELISA Kit is composed of two key components:

1) Solid microwells pre-coated with monoclonal anti TSH antibody;
2) Liquid conjugates composed of polyclonal anti-TSH conjugated with horse radish peroxidase (HRP-TSH conjugates).

The sensit TSH is a solid phase competitive ELISA. The samples, TSH Antibody-Biotin Solution and the diluted TSH enzyme conjugate are added to the wells coated with Streptavidin. TSH in the patient's serum competes with a TSH enzyme (HRP) conjugate for binding sites. Unbound TSH and TSH enzyme conjugate is washed off by wash buffer during a wash step. Upon the addition of the substrate, the intensity of color is inversely proportional to the concentration of TSH in the samples. A standard curve generated relating color intensity to the concentration of the TSH

REAGENTS & MATERIALS PROVIDED

Item	Description	Quantity
1.	Microwell coated with Sterptavidin	12 strips x 8 wells×1
2.	TSH Standard : 6 vials (ready to use)	0.5 mL
3.	TSH- HRP conjugate	0.7mL
4.	Anti- TSH Biotim Solution	7 mL
5.	Assay Diluent: 1 bottle	7 mL
6.	TMB Substrate: 1 bottle (ready to use)	12 mL
8.	Stop Solution: : 1 bottle (ready to use)	12 mL
10.	20 x Wash concentrate : 1 bottle	25 ml

Materials and reagents required but not provided in the kit

1) 50µl and 100µl volume pipette with precision better than 1.5%.
2) Distilled or De-ionized water
3) Microplate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable.
4) Absorbent paper for blotting the microplate wells.
5) Parafilm or other adhesive film for sealing the plate.
6) Timer
7) Incubator

STORAGE & STABILITY

Return all reagents requiring refrigeration immediately after use. All reagents except the concentrated wash buffer are ready to use as supplied. Reseal the microwells after removing the desired number of wells. All the reagents are stable through the expiration date printed on the label if not opened. Store the test kit over 8°C till the expiration date indicated on the pouch / carton. DO NOT FREEZE. Ensure that the test device is brought to room temperature before opening.

PRECAUTIONS & WARNING

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 devices.
3) Bring all reagents to room temperature (18°C-28°C) before use.
4) Do not use hemolized blood specimen for testing.
5) Do not use the components in any other type of test kit as a substitute for the components in this kit.
6) In the beginning of each incubation and after adding Stopping Solution, gently rocking the microwells to ensure thorough mixing. Avoid the formation of air bubbles as which results in inaccurate absorbance values. Avoid splash liquid while rocking or shaking the wells
7) Don't allow the microplate to dry between the end of the washing operation and the reagent distribution.
8) Do not ingest the reagents. Avoid contact with eyes, skin and mucose. Wear protective clothing and disposable gloves while handling the kit reagents and clinical specimens. Wash hands thoroughly after performing the test.
9) Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
10) Users of this test should follow the US CDC Universal Precautions for prevention of transmission of blood-borne pathogens.
11) Use a new distribution tip for each specimen. Never use the specimen container to distribute conjugate and substrate.
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 substrate solution.
13) The substrate solution must be colorless. The appearance of color indicates that the reagent cannot be used and must be replaced. The Substrate B must be stored in the dark.
14) The wash procedure is critical. Wells must be aspirated completely before adding the Washing Solution or liquid reagents. Insufficient washing will result in poor precision and falsely elevated absorbance.
15) Avoid strong light during color development.
16) Dispose of all specimens and materials used to perform the test as biohazardous waste.

SAMPLE COLLECTION & PREPARATION

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

REAGENT PREPARATION

1) Bring all reagents, controls to room temperature (18°C-28°C).
2) Dilute concentrated Wash Buffer 30X with water as following:

Plate	DI water	30X wash buffer	Final Volume
Full plate	290 mL	10m L	300 mL
Half plate	145 mL	5 mL	150 mL
A quarter plate	72.5 mL	2.5 mL	75 mL

If precipitant appears, warm up the concentrated wash buffer at 37°C.

3) Reagents should be mixed well before adding to the test wells.
4) Mark on the ELISA Working sheet with appropriate information after determining the number of microwells needed. Positive and Negative Controls require to be run in duplicate to ensure accuracy.

ASSAY PROCEDURE

1) Remove the desired number of strips and secure them in the microwell F rame. Reseal un-used strips.
2) Add specimens according to the designation on the ELISA Working Sheet
   - 2.1 Blank wells: Leave the blank wells alone (2 wells). Don't add any reagents.
   - 2.2 Control wells: Add 50 µL of TSH Positive Control (2 wells), Negative Control (2 wells) into the designated control wells, respectively.
   - 2.3 Test wells: Add 50 µL of test specimens into each test well, respectively.

   To ensure better precision, use pipette to handle solution.
3) Add 50 µL of the TSH conjugates to each well, except the blank well.

4) Gently rock the wells for twenty second, then cover the wells.
5) Incubate the wells at 37 °C for to 90 minutes.
6) Carefully remove the incubation mixture by empting the solution into a waste container. Fill each well with diluted wash buffer (350 µL per well) and shake gently for 20-30 seconds. Discard the wash solution completely and tapping the plate on absorbent paper. Repeat above procedure 4 more times.
7) Add 100 µL of TMB substrate into each well including the blank well.
8) Incubate at 37 °C in dark for 20 minutes.
9) Stop the reaction by adding 100 µL of stop solution to each well. Gently mix for 20-30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
10) Set the microplate reader wavelength at 450 nm and measure the absorbance (OD) of each well against the blank 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.

FLOW CHART OF ASSAY PROCEDURE

1)	Secure strips in microwell frame	Number of strips
2)	Add controls or specimen	50 µL
3)	Add conjugate gently rock	50 µL 20 seconds
4)	Incubate	37◦C, 90 minutes
5)	Wash: manual or automatic	5 times 350 µL/well
6)	Add TMB substrate . Gently rock	100 µL 20 seconds
7)	Incubate	37◦C, 20 minutes
8)	Add Stop solution. Gently rock	100 µL 20 seconds
9)	Read result	450/620-690nm Within 15 minutes

INTERPRETATION OF RESULTS

A. Set up the cut-off value

The cutoff value = 0.13 + NC
NC: Mean OD value of Negative Control.

B. Calculation of specimen OD ratio

Calculate an OD ratio for each specimen by dividing its OD value by the Cut-off Value as follows:

$$\text{Specimen OD ratio} = \frac{\text{Specimen OD}}{\text{Cut off Value}}$$

C. Assay validation

The mean OD value of the Blank should be ≤ 0.08
The mean OD value of the TSH positive controls should be ≥ 0.50. The mean OD value of the TSH negative controls should be ≤ 0.10.
If above specification are not met, the assay is Invalid. Check the assay procedure including incubation time and temperature and repeat assay.

D. Interpretation of the results

Specimen OD ratio	
Negative	< 1.00
Positive	≥ 1.00

1) The negative result indicates that there is no detectable TSH in the specimen
2) Results just below the cut-off value (Lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to retest in duplicate the corresponding specimens when it is applicable).
3) Specimens with cut-off ≥ 1 are initially considered to be positive by the Sensit TSH ELISA kit. They should be retested in duplicate before the final interpretation.
If after re-testing of a specimen, the absorbance value of the 2 duplicates are less than the cut-off value, the initial result is non repeatable and the specimen is considered to be negative with the Sensit TSH ELISA Kit.
Non repeatable reactions are often caused by:
- Inadequate microwell washing,
- Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.)
- Contamination of negative specimens by serum or plasma with a high antibody titer,
- Contamination of the stopping solution

If after re-testing the absorbance of one of the duplicates is equal or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the Sensit TSH ELISA Kit, subject to the limitation of the procedure, described below.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

Analytical sensitivity of the assay has been estimated at 0.1ng/ml for TSH Ad as well as TSH Ay, the results is presented as the minimum detection limit when the standards spiked into 20 different negative specimens.

Clinical Performance

A total of 1033 patient specimens from suspectible subjects were tested by the Sensit HBs Ag ELISA kit. Comparison for all the subjects is showed in the following table:

Ref TSH ELISA	Positive	Negative	Total
Positive	105	0	105
Negative	2	926	928
Total	107	926	1033

Relative Sensitivity: 100%, Relative Specificity :99.78%, Overall Agreement: 99.81%

Precision

Intra-assay precision was determined by assaying 20 replicated of three negatives, three weak positives and three strong positives.

Specimens	No. of Specimens	No. of replicates	CV
Negatives	3	20	5.6-20%
High Positives	3	20	3.0-5.9%
Low positives	3	20	6.4-11.4%

Cross reactivity

No false positive TSH ELISA test results were observed on 10 positives specimens from each of the following disease states or special conditions, respectively:

HIV HCV Syphilis Dengue Malaria Typhoid

Inference

Common substances (such as pain and fever medication and blood components) may affect the performance of the Sensit TSH ELISA Kit. Interference was studied by spiking these substances into 3 TSH clinical specimens: negative, low positive and high positive. The results demonstrate that at the concentrations tested, the substances studied do not affect the performance of the Sensit TSH ELISA Kit.

List of potentially interfering substances and concentrations tested:

1. Salicylic acid 4.34mmol/L
2. EDTA 3.4 umol/L
3. Glucose 55mmol/L
4. Sodium citrate 1.3%
5. Heparin 3.000 U/L
6. Bilirubin 10 mg/dL
7. Creatinine 442umol/L

LIMITATION OF THE TEST

1. The Assay Procedure and the Assay Result Interpretation must be followed closely when testing the presence of TSH in serum or plasma from individual subjects. Failure to follow the procedure may give inaccurate results.
2. The Sensit TSH ELISA Kit is limited to the qualitative detection of TSH at a sensitivity level of 0. 1 ng/mL in human serum or plasma. The intensity of color does not have linear correlation with the antigen titer in the specimen.
3. A negative result for an individual subject indicates absence of detectable TSH. However, a negative test result does not preclude the possibility of exposure to or infection with HBV.
4. A negative result can occur if the quantity of TSH present in the specimen is below the detection limits of the assay (below 0.1 ng/mL), or the TSH that are detected are not present during the stage of disease in which a specimen is collected.
5. Some specimens containing unusually high titer of heterophile antibodies or rheumatoid factor may affect expected results.
6. The results obtained with this test should only be interpreted in conjunction with other diagnostic procedures and clinical findings.

REFERENCES

1) Frank JE; Faix JE; Hermos RJ; Mullaney DM; Rojan DA; Mitchell ML; Klein RZ Thyroid function in very low birth weight infants: effects on neonatal hypothyroidism screening. J Pediatr 1996;128(4):548-54.
2) Thakur C; Saikia TC; Yadav RN. 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.

Key to symbols used			
	Manufacturer		Expiration/use by date
	Do not reuse		Date of manufacture
	Consult IFU [Instructions For Use]	LOT	Batch code
	Temperature limitation 2-30°C	IVD	In Vitro diagnostic medical device
	Contains sufficient for 'X ' kits		Do not use if package is damaged
	Keep dry	REF	Catalogue No

Manufactured by,

ubio Biotechnology Systems Pvt Ltd.
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