AccuDiag™ TSH ELISA Kit

REF 3122-16

IVD See External Label 2°C-8°C 96 Tests

TSH ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0-40 µIU/mL
Sample	50 µL serum
Sensitivity	0.2 µIU/mL
Incubation Time	80 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

Enzyme Immunoassay for the Quantitative Determination of Thyroid Stimulating Hormone (TSH) in Human Serum.

INTENDED USE

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

SIGNIFICANCE AND SUMMARY

The determination of serum or plasma levels of thyroid stimulating hormone (TSH) is recognized as a sensitive method in the diagnosis of primary and secondary hypothyroidism. TSH 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 a 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 less TSH is secreted by the pituitary. 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 hormonee (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 a monoclonal antibody in this TSH ELISA test 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.

ASSAY PRINCIPLE

The TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used for solid phase (microtiter wells) immobilization and a goat anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 60-minute incubation at room temperature, the wells are washed to remove unbound labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 2N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only.

REAGENTS

Materials provided with the test kit

1. Antibody-coated microtiter wells 96 wells
2. Enzyme Conjugate Reagent 12 ml
3. TMB Substrate 12 ml
4. Stop Solution 12 ml
5. Set of Reference Standards: 1.0 ml
   (0, 0.5, 2, 5, 10, and 40µIU/ml, in liquid form (ready to use) or lyophilized form)
6. Wash buffer Concentrate (50X), 15 ml
7. Control Set (optional)

Materials required but not provided

1. Precision pipettes: 40µl-200µl, 200-1000µl
2. Disposable pipette tips
3. Distilled waterVortex mixer or equivalent
4. Absorbent paper or paper towel
5. Graph paper

6. Microtiter plate reader

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-22°C) before use.
2. If reference standards are lyophilized, reconstitute each standard with 0.5 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.
3. Dilute 1 volume of Wash Buffer Concentrate (50x) with 49 volumes of distilled water. For example, dilute 15 ml of Wash Buffer Concentrate (50x) into 735 ml of distilled water to prepare 750 ml of washing buffer (1x). Mix well before use.

STORAGE OF THE KIT AND INSTRUMENTATION

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.
3. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2.5 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 50µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to have a complete mixing in this step.
5. Incubate at room temperature (18-22°C) for about 60 minutes.
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Rinse and flick the microtiter wells 5 times with wash buffer (1x).
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100µl of TMB solution into each well. Gently mix for 5 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100µl of Stop Solution to each well.
12. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read optical density at 450nm with a microtiter well reader within 15 minutes.

Important Note:
The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

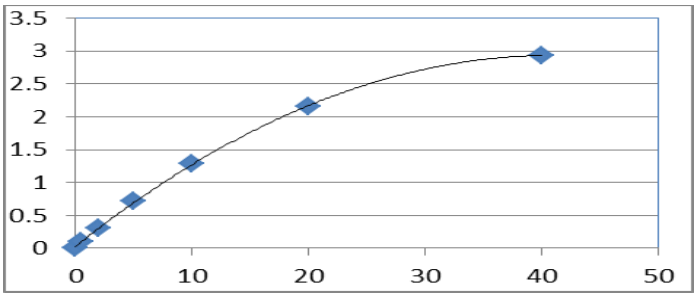
RESULTS

Calculate the mean absorbance value (A_{450}) for each set of reference standards, specimens, controls and patient samples. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in µIU/ml on graph paper, with absorbance values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the mean absorbance values for each specimen to determine the corresponding concentration of TSH in µIU/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density reading at 450nm shown in the Y axis against TSH concentrations shown in the X axis. This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each user should obtain his or her own data and standard curve.

TSH (µIU/ml)	Absorbance (450nm)
0	0.010
0.5	0.075
2	0.235
5	0.540
10	1.004
40	2.801



EXPECTED VALUES AND SENSITIVITY

Based on a study of 139 random normal adult blood samples, nonmal TSH values and ranges (in µIU/ml) were shown in the following table.

Expected Values		2.5 Percentile (70% Conf Int)	
Low Normal	0.39	Low Range	0.28-0.53
High Normal	6.16	High Range	5.60-6.82

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent on a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. Therefore, each laboratory should depend on the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

The minimum detectable concentration of TSH by this assay is estimated to be 0.2 µIU/ml.

LIMITATIONS OF PROCEDURE

There are some limitations of the assay:

1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

2. Studies have implicated possible interference in immunoassay results in some patients with known rheumatoid factor and antinuclear antibodies. Serum samples from patients who have received infusions containing mouse monoclonal antibodies for diagnostic or therapeutic purposes, may contain antibody to mouse protein (HAMA). Although we have added some agents to avoid the interferences, we cannot guarantee it will eliminate all the effects of that.

REFERENCES

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4. Burger H.G. and Patel Y.C. Thyrotropin releasing hormone-TSH **Clinic. Endocrinol. and Metab.** 1977; 6: 831
5. Snyder P.J. and Utiger R.D. **J. Clin. Endocrinol. Metab.** 1972; 34: 380-385

MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
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Date Adopted	**2022-09**
REF **3122-16**	**AccuDiag™ - TSH ELISA**
Brand Name	**AccuDiag™**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands.** **www.cepartner4u.eu**
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