



AccuDiag™

TSH

ELISA Kit

REF 3122

PIC TH3122YR1

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

TSH ELISA

Method	Enzyme Linked Immunosorbent Assay
Principle	Sandwich Complex
Detection Range	0-25 µIU/mL
Sample	100 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

INTENDED USE

For the quantitative determination of thyroid stimulating hormone (TSH) concentration in human serum. The assay is useful in the diagnosis of thyroid or pituitary disorders.

SIGNIFICANCE AND SUMMARY

(TSH or thyrotropin) is recognized as a sensitive method in the diagnosis of primary and secondary hypothyroidism.¹ TSH is secreted by the anterior lobe of the pituitary gland and induces the production and release of thyroxine (T₄) and triiodothyronine (T₃) from the thyroid gland.² It is a glycoprotein with a molecular weight of approximately 28,000 daltons, consisting of two chemically different subunits, alpha and beta.³

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.^{4,5} TSH and the pituitary glycoproteins: luteinizing hormone (LH), follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG), have identical alpha chains.² The beta chains are distinct but do contain regions with identical amino acid sequences. These regions of homology can cause considerable cross-reactivity with some polyclonal TSH antisera. The use of a monoclonal antibody in Diagnostic Automation, Inc. 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.^{6,7,8}

ASSAY PRINCIPLE

The Diagnostic Automation, Inc. TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay.^{9,10} 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 immobilization (microtiter wells), and 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 a 60-minute or overnight incubation at room temperature, the solid phase is washed with water to remove unbound labeled antibodies. A solution of 3, 3', 5, 5'-Tetramethylbenzidine (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 1N HCl, and the resulting yellow color is 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

- Murine Monoclonal Anti-TSH-coated microtiter wells.
- Set of Reference Standards: 0, 0.5, 2, 5, 10 and 25 µIU/ml, lyophilized.
- Enzyme Conjugate Reagent, 13 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Materials required but not provided

- Precision pipettes: 100 µl and 1.0 ml.
- Disposable pipette tips.
- Distilled water.
- Vortex mixer or equivalent.
- Absorbent paper or paper towel.

Diagnostic Automation/Cortez Diagnostics, Inc.

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- Graph paper.
- Microtiter plate reader

REAGENT PREPARATION

1. All reagents should be brought to room temperature (18-25°C) before use.
2. Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. Reconstituted standards will be stable for up to 30 days when stored sealed at 2-8°C.

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.

STORAGE AND STABILITY

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. Opened test kits will remain stable until the expiration date shown, provided it is stored as described above. A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-2 OD or greater at 450 nm wavelength is acceptable for use in absorbance measurement.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards, specimens, and controls into appropriate wells.
3. Dispense 100 µl of Enzyme Conjugate Reagent into each well.
4. Thoroughly mix for 30 seconds. It is very important to mix them completely.
5. Incubate at room temperature (18-25°C) for 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 distilled or deionized water. (Please do not use tap water.)
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100 µl of TMB Reagent into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop the reaction by adding 100 µl 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 absorbance at 450 nm with a microtiter well reader **within 15 minutes.**

RESULTS

1. Calculate the mean absorbance value (A_{450}) for each set of reference standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance obtained

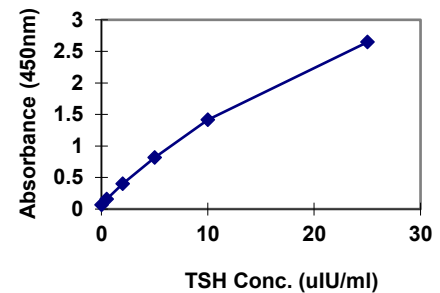
from each reference standard against its concentration in µU/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.

3. Use the mean absorbance values for each specimen to determine the corresponding concentration of TSH in µU/ml from the standard curve.

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm 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	0.063
0.5	0.157
2.0	0.398
5.0	0.818
10.0	1.415
25.0	2.645



EXPECTED VALUES

The mean TSH values based on 160 random normal adult blood samples, is 1.6 (0.4-6.0) µIU/ml. TSH levels exceeding 10 µIU/ml, suggest primary hypothyroidism. Low or undetectable TSH levels may be normal, but may also indicate secondary hypothyroidism (insufficient secretion of TSH or TRH). Low levels may also be due to hyper-secretion of T-3 and T-4 due to Grave's disease or thyroiditis. Differential diagnosis is best achieved by simultaneous determination of TSH and free T-4 levels in serum.

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

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.




- Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
- For professional use only. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016



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