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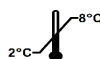
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**Instructions for use**  
**TSH ELISA 2nd Generation**

**REF**

**TF E-2000**



**IVD**

**CE**

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## **1. INTRODUCTION**

### **1.1 Intended Use**

The **TSH ELISA** is an enzyme immunoassay for the quantitative *in vitro diagnostic* measurement of TSH in serum or heparin plasma.

### **NOT INTENDED FOR NEWBORN SCREENING.**

### **1.2 Summary and Explanation**

Measurement of the serum concentration of thyrotropin (TSH), a glycoprotein with a molecular weight of 28,000 daltons and secreted from the anterior pituitary, is generally re-garded as the most sensitive indicator available for the diagnosis of primary and secondary (pituitary) hypothyroidism (1,2). Increase in serum concentrations of TSH, which is primarily responsible for the synthesis and release of thyroid hormones, is an early and sensitive indicator of decrease thyroid reserve and in conjunction with decreased thyroxine (T4) concentrations is diagnostic of primary hypothyroidism. The expected increase in TSH concentrations demonstrates the classical negative feedback system between the pituitary and thyroid glands. That is, primary thyroid gland failure reduces secretion of the thyroid hormones, which in turn stimulates the release of TSH from the pituitary.

Additionally, TSH measurements are equally useful in differentiating secondary and tertiary (hypothalamic) hypothyroidism from the primary thyroid disease. TSH release from the pituitary is regulated by thyrotropin releasing factor (TRH), which is secreted by the hypothalamus, and by direct action of T4 and triiodothyronine (T3), the thyroid hormones, at the pituitary. Increase levels of T3 and T4 reduces the response of the pituitary to the stimulatory effects of TRH. In secondary and tertiary hypothyroidism, concentrations of T4 are usually low and TSH levels are generally low or normal. Either pituitary TSH deficiency (secondary hypothyroidism) or insufficiency of stimulation of the pituitary by TRH (tertiary hypothyroidism) causes this. The TRH stimulation test differentiates these conditions. In secondary hypothyroidism, TSH response to TRH is blunted while a normal or delayed response is obtained in tertiary hypothyroidism.

Further, the advent of immunoenzymometric assays has provided the laboratory with sufficient sensitivity to enable the differentiating of hyperthyroidism from euthyroid population and extending the usefulness of TSH measurements. This method is a second-generation assay, which provide the means for discrimination in the hyperthyroid-euthyroid range.

## **2. PRINCIPLE OF THE TEST**

The TSH ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal [mouse] antibody directed towards a unique antigenic site of the TSH molecule. An aliquot of patient sample containing endogenous TSH is incubated in the coated well with enzyme conjugate, which is an anti-TSH antibody conjugated with horseradish peroxidase. After incubation the unbound conjugate is washed off.

The amount of bound peroxidase is proportional to the concentration of TSH in the sample.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of TSH in the patient sample.

## **3. WARNINGS AND PRECAUTIONS**

1. This kit is for *in vitro* diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of the instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 °C to 8 °C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

9. Allow the reagents to reach room temperature (21 °C to 26 °C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with *Stop Solution* containing 0.5 M H<sub>2</sub>SO<sub>4</sub>. It may cause skin irritation and burns.
18. Some reagents may contain Proclin, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundant volume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

#### 4. REAGENTS

##### 4.1 Reagents provided

**TF E-2031**    **96**                      **Microtiterwells**  
 Content:     12 x 8 (break apart) strips, 96 wells;  
                  Wells coated with anti-TSH antibody (monoclonal)


**Standards and Controls** - Ready to use

Cat. no.	Component		Concentration	Volume/ Vial
<b>TF E-2001</b>	<b>STANDARD</b> A	Standard A	0 mIU/l	0.4 ml
<b>TF E-2002</b>	<b>STANDARD</b> B	Standard B	0.25 mIU/l	0.4 ml
<b>TF E-2003</b>	<b>STANDARD</b> C	Standard C	0.75 mIU/l	0.4 ml
<b>TF E-2004</b>	<b>STANDARD</b> D	Standard D	2.0 mIU/l	0.4 ml
<b>TF E-2005</b>	<b>STANDARD</b> E	Standard E	5.0 mIU/l	0.4 ml
<b>TF E-2006</b>	<b>STANDARD</b> F	Standard F	15 mIU/l	0.4 ml
<b>TF E-2051</b>	<b>CONTROL</b> 1	Control Low	For control values and ranges please refer to vial label or QC-Datasheet.	0.4 ml
<b>TF E-2052</b>	<b>CONTROL</b> 2	Control High		0.4 ml

Content:     The standards are calibrated against WHO International Standard for TSH IRP (81/565);  
 Contain preservative.

**TF E-2040**    **CONJUGATE**                      **Enzyme Conjugate** - Ready to use  
 Content:     Anti-TSH antibody conjugated to horseradish peroxidase;  
                  Contains preservative.  
 Volume:     1 x 12 ml

**TF E-0055**    **SUBSTRATE**                              **Substrate Solution** - Ready to use  
 Content:     Tetramethylbenzidine (TMB).  
 Volume:     1 x 12 ml

**FR E-0080** **STOP-SOLN** **Stop Solution** - Ready to use  
Content: contains 0.5 M H<sub>2</sub>SO<sub>4</sub>,  
Avoid contact with the stop solution. It may cause skin irritations and burns.  
Volume: 1 x 14 ml  
Hazards identification:   
H290 May be corrosive to metals.  
H314 Causes severe skin burns and eye damage.

**TF E-0030** **WASH-CONC** **40x** **Wash Solution** - 40X concentrated  
Volume: 1 x 25 ml  
see „Preparation of Reagents“.

**Note:** Additional *Standard A* for sample dilution is available upon request.

#### 4.2 Materials required but not provided

- A microtiter plate calibrated reader (450 ± 10 nm)
- Calibrated variable precision micropipettes
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

#### 4.3 Storage Conditions

When stored at 2 °C to 8 °C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.

Opened reagents must be stored at 2 °C to 8 °C. Microtiter wells must be stored at 2 °C to 8 °C. Once the foil bag has been opened, care should be taken to close it tightly again. Opened kits retain activity for two months if stored as described above.

#### 4.4 Reagent Preparation

Bring all reagents and required number of strips to room temperature prior to use.

##### **Wash Solution**

Add deionized water to the 40X concentrated Wash Solution.

Dilute 25 ml of concentrated *Wash Solution* with 975 ml deionized water to a final volume of 1000 ml.

*The diluted Wash Solution is stable for 2 weeks at room temperature.*

#### 4.5 Disposal of the Kit

The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Safety Data Sheet.

#### 4.6 Damaged Test Kits

In case of any severe damage to the test kit or components, the manufacturer has to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

### 5. SPECIMEN COLLECTION AND PREPARATION

Serum or heparin plasma can be used in this assay.

For accurate comparison to established normal values, a fasting morning serum sample should be obtained.

Do not use haemolytic, icteric or lipaemic specimens.

Please note: Samples containing sodium azide should not be used in the assay.

#### 5.1 Specimen Collection

##### **Serum:**

Collect blood by venipuncture (e.g. Sarstedt Monovette for Serum), allow to clot, and separate serum by centrifugation at room temperature. Do not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

**Plasma:**

Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

**5.2 Specimen Storage and Preparation**

Specimens should be capped and may be stored for up to 5 days at 2 °C to 8 °C prior to assaying.

Specimens held for a longer time (up 30 days) should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

**5.3 Specimen Dilution**

If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard A* and reassayed as described in Assay Procedure.

For the calculation of the concentrations this dilution factor has to be taken into account.

Example:

a) dilution 1:10: 10 µl sample + 90 µl Standard A (mix thoroughly)

b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl Standard A (mix thoroughly).

**6. ASSAY PROCEDURE****6.1 General Remarks**

- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.

**6.2 Test Procedure**

Each run must include a standard curve.

<b>1.</b> Secure the desired number of Microtiter wells in the frame holder.
<b>2.</b> Dispense <b>25 µl</b> of each <b>Standard, Control</b> and <b>sample</b> <u>with new disposable tips</u> into appropriate wells.
<b>3.</b> Incubate for <b>10 minutes</b> at room temperature.
<b>4.</b> Dispense <b>100 µl Enzyme Conjugate</b> into each well. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
<b>5.</b> Incubate for <b>90 minutes</b> at room temperature.
<b>6.</b> Briskly shake out the contents of the wells. Rinse the wells <b>5 times</b> with diluted <i>Wash Solution</i> (300 µl per well). Strike the wells sharply on absorbent paper to remove residual droplets. <b>Important note:</b> The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
<b>7.</b> Add <b>100 µl</b> of <b>Substrate Solution</b> to each well.
<b>8.</b> Incubate for <b>20 minutes</b> at room temperature.
<b>9.</b> Stop the enzymatic reaction by adding <b>100 µl</b> of <b>Stop Solution</b> to each well.
<b>10.</b> Determine the absorbance (OD) of each well at <b>450 ± 10 nm</b> with a microtiter plate reader. It is recommended that the wells be read <b>within 5 minutes</b> after adding the <i>Stop Solution</i> .

Preferably readings should take place immediately after stopping the reaction since the OD<sub>450 nm</sub> may slightly decrease with the course of time.

### 6.3 Calculation of Results

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using semi-logarithmic graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.
5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 15 mIU/l. For the calculation of the concentrations this dilution factor has to be taken into account.

#### 6.3.1 Example of Typical Standard Curve

The following data is for demonstration only and **cannot** be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard A (0 mIU/l)	0.01
Standard B (0.25 mIU/l)	0.04
Standard C (0.75 mIU/l)	0.11
Standard D (2.0 mIU/l)	0.32
Standard E (5.0 mIU/l)	0.81
Standard F (15.0 mIU/l)	2.27

### 7. EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.

The German guideline for thyroid diagnostics recommends a normal range from 0.3 to 4.0 mIU/l

A correlation study between Abbott Architect system parameter TSH and TSH ELISA Test system with 70 specimens shows a normal range for the TSH ELISA Test system of 0.5 to 5.0 mIU/l (Abbott: 0.35 to 4.94 mIU/l)

Serum thyrotropin concentration is dependent upon a multiplicity of factors: hypothalamus gland function, thyroid gland function, and the responsiveness of pituitary to TRH. Thus, thyrotropin concentration alone is not sufficient to assess clinical status.

Genetic variations or degradation of intact TSH into subunits may affect the binding characteristics of the antibodies and influence the final result. Such samples normally exhibit different results due to the reactivity of the antibodies involved.

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

### 8. QUALITY CONTROL

Good laboratory practice requires that controls be run with each standard curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.

It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.

The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.

It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.

Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or the manufacturer directly.

## 9. PERFORMANCE CHARACTERISTICS

### 9.1 Assay Dynamic Range

The range of the assay is between 0.06 – 15 mIU/l.

### 9.2 Specificity of Antibodies (Cross Reactivity)

The cross-reactivity of the TSH ELISA method to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations.

No cross-reactions were found when testing up to

100,000 mIU/ml	Chorionic Gonadotropin (hCG)
100 mIU/ml	Follicle Stimulating Hormone (hFSH)
100 mIU/ml	Luteinizing Hormone (hLH)

### 9.3 Sensitivity

The analytical sensitivity of the TSH ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the Standard A and was found to be 0.06 mIU/l.

### 9.4 Reproducibility

#### 9.4.1 Intra Assay

The within assay variability is shown below:

Sample	n	Mean (mIU/l)	CV (%)
1	18	0.95	3.88
2	18	3.26	3.16
3	18	8.79	3.36

#### 9.4.2 Inter Assay

The between assay variability is shown below:

Sample	n	Mean (mIU/l)	CV (%)
1	4	1.18	9.17
2	4	3.29	5.33
3	4	9.05	3.32

### 9.5 Comparison Studies

Correlation between Abbott Architect system parameter TSH and TSH ELISA Test system

		Abbott Architect (TSH)	
		pos	neg
TSH ELISA test	pos	17	0
	neg	7	46

n : 70  
sensitivity : 70,8%  
specificity : 100%  
pos. PDW : 100%  
neg. PDW : 86,8%

## 10. LIMITATIONS OF USE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

### 10.1 Interfering Substances

Haemoglobin (up to 4 mg/ml), Bilirubin (up to 0.5 mg/ml) and Triglyceride (up to 30 mg/ml) have no influence on the assay results.

### 10.2 Drug Interferences

Serum thyrotropin values may be elevated by pharmacological intervention. Domperidone, amiodazon, iodide, phenobarbital, and phenytoin have been reported to increase TSH levels.

A decrease in thyrotropin values has been reported with the administration of propranolol, methimazol, dopamine and d-thyroxine (4).

### 10.3 High-Dose-Hook Effect

No hook effect was observed in this test up to 2000 mIU/l of TSH.

## 11. LEGAL ASPECTS

### 11.1 Reliability of Results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact the manufacturer.

### 11.2 Therapeutic Consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point "Reliability of Results". Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutic consequences.

### 11.3 Liability












Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.

Claims submitted due to customer misinterpretation of laboratory results subject to point "Therapeutic Consequences". are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

## 12. REFERENCES / LITERATURE

1. Barker, S.B., "Determination of Protein Bound Iodine." *Journal Biological Chemistry*, 173, 175, (1948).
2. Chopra, I.J., Solomon, D.H., and Ho, R.S., "A Radioimmunoassay of Thyrotropin," *J. Clinical Endocrinology*, 33, 865 (1971).
3. Young, D.S., Pestaner, L.C., and Gilberman, U., "Effects of Drugs on Clinical Laboratory Tests." *Clinical Chemistry*, 21, 3660 (1975).
4. Spencer, CA, et al., "Clinical Chemistry, Interlaboratory/Intermethod differences in Functional Sensitivity of Immunometric Assays of Thyrotropin (TSH) and Impact on Reliability of Measurement of Subnormal Concentrations of TSH", 41, 367 (1995).

### Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date		Batch code		For in-vitro diagnostic use only!
	Consult instructions for use		Content		CE labelled
	Caution		Catalogue number		