Instructions for Use

Thyroid Stimulating Hormone (TSH) ELISA

IVD

CE

REF **EIA-1782**

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Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

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For In Vitro Diagnostic Use Only

Store at 2 °C to 8 °C.

1 INTRODUCTION

1.1 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.

1.2 Introduction

The determination of serum or plasma levels of thyroid stimulating hormone (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 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 (6,7,8).

2 PRINCIPLE OF THE TEST

The 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 1 N 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.

3 REAGENTS AND MATERIALS PROVIDED

1. **Antibody-Coated Wells** (1 plate, 96 wells)
   Microtiter wells coated with mouse monoclonal anti-TSH.
2. **Enzyme Conjugate Reagent** (1 dropper bottle, 13 mL)
   Contains goat anti-TSH conjugated to horseradish peroxidase.
3. **Reference Standard Set** (1 mL/vial)
   Contains 0, 0.5, 2.0, 5.0, 10.0 and 25.0 µIU/mL (WHO, 2nd IRP, 80/558) TSH in equine serum with preservatives. Lyophilized.
   See instructions for reconstitution under Reagent Preparation.
4. **TMB Reagent** (1 bottle, 11 mL)
   Contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
5. **Stop Solution** (1 N HCl) (1 bottle, 11 mL)
   Contains diluted hydrochloric acid.

4 MATERIALS REQUIRED BUT NOT PROVIDED

1. Deionized water
2. Precision pipettes: 50 µL, 100 µL, 200 µL, and 1 mL
3. Disposable pipette tips
4. Microtiter well reader capable of reading absorbance at 450 nm.
5. Orbital motion microtiter well shaker, capable of shaking at a speed of 175 ± 25 rpm
6. Absorbent paper
7. Graph paper (semi-log, etc.)
8. Quality control material (e.g., BioRad Lyphochek Control sera)

5 WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this kit tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum samples, should be considered potentially infectious. Handling and disposal should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists.
2. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
3. Do not use the reagent when it becomes cloudy or contamination is suspected.
4. Do not use the reagent if the vial is damaged.
5. Replace caps on reagents immediately. Do not switch caps.
6. Each well can be used only once.
7. Do not pipette reagents by mouth.
8. Solutions containing additives or preservatives, such as sodium azide, should not be used in the enzyme reaction.
9. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
10. For in vitro diagnostic use.

6 STORAGE CONDITIONS

Store the unopened kit at 2 °C - 8 °C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.

The opened and used reagents are stable until the expiration date if stored properly at 2 °C - 8 °C.

Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

7 INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

8 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. Avoid grossly hemolytic, lipemic, or turbid samples.

Specimens should be capped and may be stored for up to 48 hours at 2 °C - 8 °C prior to assaying. Specimens held for a longer time should be frozen only once at -20 °C prior to assay. Thawed samples should be inverted several times prior to testing.

9 REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18 °C - 25 °C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
3. Reconstitute each lyophilized standard with 1.0 mL deionized H_20. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2 °C - 8 °C, and are stable at 2 °C - 8 °C for at least 30 days.
4. Specimens expected to have a TSH concentration greater than 25 µIU/mL should be diluted 1:10 with TSH-free serum (Zero calibrator).

10 PROCEDURAL NOTES

1. Manual Pipetting:
   It is recommended that no more than 32 wells be used for each assay run. Pipetting of all standards, samples, and controls should be completed within 3 minutes.
2. Automated Pipetting:
   A full plate of 96 wells may be used in each assay run. However, it is recommended that pipetting of all standards, samples, and controls be completed within 3 minutes.
3. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
4. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

11 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 have complete mixing.
5. Incubate at room temperature (18 °C – 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 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. ***Ensure that all of the blue color changes completely to yellow.***
13. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

12 CALCULATION OF RESULTS

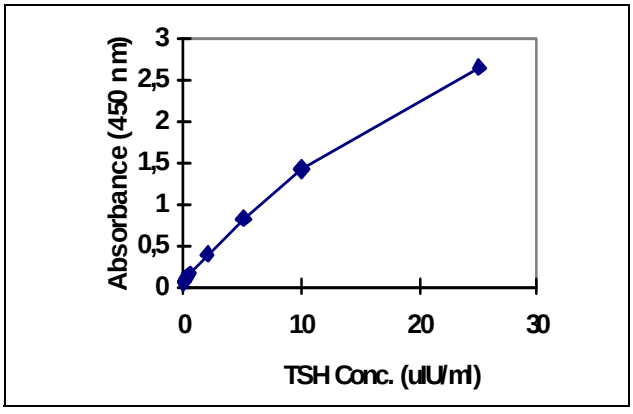
1. Calculate the mean absorbance value (OD_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in µIU/mL, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of TSH in µLU/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

12.1 Example of Standard Curve

Results of a typical standard run of the assay are shown below. The standard curve is for illustration only, and should not be used to calculate unknowns. The standard curve covers a dynamic range from 0 to 25 µIU/mL.

The absorbance (450 nm) value can be varied due to incubation at different room temperatures in different laboratories.

TSH (µIU/mL)	Absorbance (450 nm)
0	0.063
0.5	0.157
2.0	0.398
5.0	0.818
10.0	1.415
25.0	2.645



13 EXPECTED VALUES

Each laboratory should establish its own normal range based on patient population. Differences in assay technique and the use of various standards may affect results.

The results provided below are based on 38 normal and 77 hyperthyroid blood specimens. The ranges were determined from the mean ± 2SD (µIU/mL TSH). These values may differ from other published data.

	Normal	Hyperthyroid
N	38	77
Mean TSH (µIU/mL)	1.44	< 0.2
Range	0.3 - 8.1	< 0.2

14 NORMAL REFERENCE RANGE

The reference ranges provided below are based on Tietz.(12)

	TSH (µU/mL)
Adults	
21-54 years	0.4-4.2
55-87 years	0.5-8.9
Pregnancy	
1st Trimester	0.3-4.5
2nd Trimester	0.5-4.6
3rd Trimester	0.8-5.2

15 PERFORMANCE CHARACTERISTICS

15.1 Accuracy

TSH ELISA (EIA-1782) has been compared to DPC's Immulite 2000 3rd Generation TSH test. The methods are similar in that they are both used for the quantitative determination of TSH in human serum, and both use TSH calibrated and labeled in µIU/mL standardized to the WHO hTSH (2nd IRP, 80/558).

A study was conducted using 39 euthyroid and 20 hypothyroid patient samples (as determined by hospital laboratory analysis). The range of samples tested was 0.4 µIU/mL to 63 µIU/mL. The comparison demonstrated good correlation with another commercial kit as shown below:

N = 59

Correlation coefficient = 0.994

Slope = 1.027

Intercept = 0.787

EIA-1782 Mean = 8.39 µIU/mL

DPC Mean = 9.41 µIU/mL

Another 77 hyperthyroid patient samples also correlated well with the DPC test kit:

	# Samples with [TSH] ≤ 0.2 µIU/mL
DPC Kit	N = 77
EIA-1782	N = 77

15.2 Sensitivity

At TSH concentrations of 0.1 µIU/mL and 0.2 µIU/mL, the interassay CVs were determined to be 11.4% and 7.9%, respectively.

15.3 Precision

a. Intra-Assay Precision

Within-run precision was determined by replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3	4
Number of Replicates	28	26	26	26
Mean TSH (µIU/mL)	0.62	1.51	15.48	26.14
Standard Deviation	0.03	0.09	0.39	0.86
Coefficient of Variation (%)	4.6%	5.7%	2.5%	3.3%

b. Inter-Assay Precision

Between-run precision was determined by replicate measurements of four different serum samples over a series of individually calibrated assays as shown below:

Serum Sample	1	2	3	4
Number of Replicates	30	24	24	24
Mean TSH (µIU/mL)	0.64	1.46	15.38	25.26
Standard Deviation	0.05	0.10	0.87	1.75
Coefficient of Variation (%)	7.6%	7.1%	5.7%	8.9%

15.4 Recovery and Linearity Studies

a. Recovery

Various patient serum samples of known TSH levels were combined and assayed in duplicate.
The mean recovery was 98.9%.

Expected Concentration (µIU/mL)	Observed Concentration (µ/mL)	% Recovery
23.94	22.92	95.7%
11.75	11.01	93.6%
5.46	5.53	101.3%
2.76	3.04	110.1%
1.48	1.39	93.9%

b. Linearity

Two patient samples were serially diluted with zero standard to determine linearity.
The mean recovery was 94.6%.

#	Dilution	Expected Conc. (µIU/mL)	Observed Conc. (µIU/mL)	% Expected
1.	Undiluted		48.39	
	1:2	24.42	22.43	91.9%
	1:4	11.21	10.45	93.2%
	1:8	5.60	5.13	91.6%
	1:16	2.80	2.87	102.5%
	1:32	1.40	1.30	92.9%
			Average = 94.4%	
2.	Undiluted		36.42	
	1:2	18.21	18.10	99.4%
	1:4	9.10	8.48	93.2%
	1:8	4.55	4.28	94.1%
	1:16	2.27	2.22	97.8%
	1:32	1.13	1.01	89.4%
			Average = 94.8%	

15.5 Specificity

The following hormones were tested for cross-reactivity:

Hormone tested	Concentration	Produced intensity to TSH (µlUmL)
HCG - (WHO 2nd IS 61/6)	100 mIU/mL 600 mIU/mL 3,500 mIU/mL 10,000 mIU/mL 200,000 mIU/mL	0 0 0 0 0
FSH - (WHO 2nd IRP-HMG)	20 mIU/mL 100 mIU/mL 200 mIU/mL	0 0 <0.2
LH - (WHO 1st IRP 68/40)	75 mIU/mL 150 mIU/mL 300 mIU/mL	0 0 <0.2
Prolactin - (WHO 1st IRP 75/504)	10 ng/mL 50 ng/mL 200 ng/mL	0 0 <0.2
hGH - (WHO 1st IRP 65/217)	10 ng/mL 50 ng/mL 200 ng/mL	0 0 <0.2

15.6 Hook Effect

No hook effect is observed in this assay at TSH concentrations up to 1,000 µIU/mL.

16 QUALITY CONTROL

Good laboratory practice requires that low, medium and high quality control specimens (controls) be run with each calibration curve to verify assay performance. To assure proper performance, a statistically significant number of controls should be assayed to establish mean values and acceptable ranges. Controls containing sodium azide should not be used.

17 STANDARDIZATION

The TSH Reference Standards are calibrated against the World Health Organization's Second International Reference Preparation of hTSH 2nd IRP-80/558).

18 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. 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.
3. Serum samples demonstrating gross lipemia, gross hemolysis, or turbidity should not be used with this test.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. The TSH assay (EIA-1782) has not been tested on newborns, and is not for use in screening newborns.
6. If the device fails to perform, use alternative diagnostic procedure or consult manufacturer.

19 REFERENCES / LITERATURE

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1 EINLEITUNG

1.1 Verwendungszweck

Enzymimmunoassay zur quantitativen Bestimmung von TSH (Thyroid stimulating hormone) in humanem Serum.

1.2 Klinische Bedeutung

TSH (thyroid stimulating hormone) ist ein Glykoprotein mit einem Molekulargewicht von ca. 28.000 Dalton. Es besteht aus zwei verschiedenen Untereinheiten, alpha und beta. Die Bestimmung von TSH oder Thyrotropin bietet eine sensitive Methode zur Diagnose einer primären oder sekundären Hypothyreose. TSH wird vom Hypophysenvorderlappen sezerniert und induziert die Produktion und Freisetzung von Thyroxin (T4) und Trijodthyronin (T3) aus der Schilddrüse. Die Sekretion des TSH reagiert empfindlich auf den Mangel oder den Überschuss der Scshilddrüsenhormone T4 und T3 und ist damit ein verlässlicher Parameter der tatsächlichen Wirkung dieser Hormone im Körpergewebe.

TSH ist sehr wichtig für die Aufrechterhaltung der normalen Schilddrüsenfunktion, obwohl es im Blut in extrem niedrigen Konzentrationen vorhanden ist. Die Freisetzung von TSH wird durch TRH (TSH-releasing hormone) reguliert, das vom Hypothalamus produziert wird. Die TSH und TRH Spiegel im Blut sind umgekehrt proportional zur Konzentration der Schilddrüsenhormone im Blut. Wenn im Blut eine hohe Konzentration an Schilddrüsenhormonen vorhanden ist, wird vom Hypothalamus weniger TRH freigesetzt und daher auch weniger TSH von der Hypophyse sezerniert. Wenn der Schilddrüsen-spiegel im Blut sinkt, tritt die umgekehrte Reaktion ein: Mehr TRH und somit auch THS werden freigesetzt bzw. produziert. Dieser negative Feedback-Mechanismus dient der Regulation des Schilddrüsen-Hormon Spiegels im Blut.

TSH und die hypophysären Glykoproteine (LH, FSH und HCG) haben identische Alpha Ketten. Die Beta Ketten sind unterschiedlich, enthalten aber Regionen mit identischer Aminosäuresequenz. Diese homologen Regionen können bei der Verwendung polyklonaler TSH Antiseren zu unerwünschten Interferenzen mit den genannten Glykoproteinen führen und so bei Frauen in der Menopause oder schwangeren Frauen zu falsch erhöhten TSH Werten führen. Der DRG TSH ELISA verwendet einen spezifischen monoklonalen Antikörper um unerwünschte Kreuzreaktionen zu vermeiden.

2 TESTPRINZIP

Der Ultrasensitive TSH ELISA basiert auf dem Prinzip des Solid Phase Enzyme-linked immunosorbent Assays. Der Test verwendet einen monoklonalen Antikörper der gegen eine bestimmte antigene Determinante des intakten TSH Moleküls gerichtet ist. Maus monoklonaler Antikörper wird zur Solid Phase Immobilisierung verwendet (auf den Wells der Mikrotiterplatte). Die Konjugatlösung enthält einen Ziegen anti-TSH Antikörper, konjugiert mit Meerrettichperoxidase. Während der Inkubation kann die Probe simultan mit den beiden Antikörpern reagieren, wobei die TSH Moleküle mit den beiden Antikörpern einen Sandwichkomplex bilden. Nach 60 Minuten Inkubation bei Raumtemperatur werden die nicht gebundenen markierten Antikörper durch einen Waschschritt entfernt. Anschließend wird die H_2O_2/TMB Substratlösung zugegeben und 20 Min. inkubiert, wobei sich eine blaue Farbe entwickelt. Die Farbentwicklung wird durch Zugabe von 3N HCL gestoppt; dabei findet ein Farbumschlag auf Gelb statt. Die TSH Konzentration ist direkt proportional zur Farbintensität der Probe. Die Extinktion wird bei 450 nm im Spektrophotometer gemessen.

3 REAGENZIEN

1. **Mikrotiter Wells**, beschichtet mit Murinem Monoklonalem Anti-TSH-Antikörper.
2. **Enzyme Conjugate**: Enzym-Konjugat Lösung, 13 mL.
3. Reference **Standard**-Set: 0, 0.5, 2, 5,10 und 25 µlU/mL, lyophilisiert.
   (WHO, 2nd IRP, 80/558)
4. **TMB Reagent**: TMB Reagenz, Substratlösung (gebrauchsfertig): 11 mL
5. **Stop Solution:** Stopplösung: 1N HCl, 11 mL

4 BENÖTIGTE MATERIALIEN, DIE NICHT IM KIT ENTHALTEN SIND

- Deionisiertes Wasser
- Präzisions-Pipette: 100 µL, 1 mL
- Einweg-Pipettenspitzen
- Mikrotiterplatten-Lesegerät (450 nm Wellenlänge)
- Vortexer
- Saugpapier oder Papierhandtuch
- Millimeterpapier (semi-log)
- Qualitätskontrollmaterial

5 LAGERUNG DER REAGENZIEN

Die ungeöffneten Reagenzien behalten bei Lagerung um 2 °C - 8 °C ihre Reaktivität bis zum Verfallsdatum. Nach dem Verfallsdatum die Reagenzien nicht mehr verwenden.

Nach dem Öffnen sollten alle Reagenzien bei 2 °C - 8 °C gelagert werden.

Die Mikrotiterwells sollten bei 2 °C - 8 °C gelagert werden. Der einmal geöffnete Folienbeutel sollte stets sehr sorgfältig wieder verschlossen werden. Unter den beschriebenen Lagerbedingungen behalten geöffnete Kits ihre Reaktivität bis zum angegebenen Verfallsdatum

6 PROBENENTNAHME UND AUFBEWAHRUNG

Blut durch Venenpunktion entnehmen, gerinnen lassen und das Serum durch Zentrifugation bei Raumtemperatur abtrennen. Für diesen Test sollten nur Serumproben ohne Zusätze verwendet werden.

Proben sollten stets gut verschlossen sein und können vor Testbeginn bis zu 48 Stunden bei 2 °C - 8 °C gelagert werden.

Für eine längere Aufbewahrung sollten die Proben eingefroren bei -20 °C bis zum Testbeginn gelagert werden. Nur einmal einfrieren. Aufgetaute Proben sollten vor Testbeginn vorsichtig durchmischt werden, ohne Schaumbildung.

7 VORBEREITUNG DER REAGENZIEN

1. Alle Reagenzien vor Gebrauch auf Raumtemperatur (18 °C – 25 °C) bringen.
2. Die **lyophilisierten Standards** jeweils mit **1,0 mL** deionisiertem Wasser rekonstituieren. Mindestens 20 Minuten stehen lassen und vorsichtig mischen.
   Rekonstituierte Standards sind bei 2 °C bis 8 °C für bis zu 30 Tagen stabil.
3. Proben mit einer zu erwartenden Konzentration > 25 µIU/mL sollten 1:10 mit TSH-freiem Serum (Nullstandard) verdünnt werden.

8 TESTDURCHFÜHRUNG

1. Die benötigte Anzahl an Wells in der Halterung befestigen.
2. 100 µL Standards, Kontrollen und Proben in die entsprechenden Wells pipettieren.
3. 100 µL Enzymkonjugat in alle Wells geben.
4. Ca. 30 Sek. gründlich mischen. Eine vollständige Vermischung der Reagenzien ist sehr wichtig!
5. 60 Minuten bei Raumtemperatur (18 °C - 25 °C) inkubieren.
6. Inkubationslösung zügig abschütten. Wells 5-mal mit deionisiertem Wasser, ca. 400 µL pro Well, waschen. Nach dem letzten Waschen Wassertropfen aus den Wells durch Ausklopfen auf saugfähigem Papier entfernen.
7. 100 µL TMB-Reagenz in alle Wells geben. 10 Sekunden vorsichtig mischen.
8. 20 Minuten bei Raumtemperatur inkubieren.
9. Die Reaktion durch Zugabe von 100 µL Stopplösung (1N HCl) stoppen.
10. 30 Sek. vorsichtig mischen. Es ist wichtig, dass der Farbumschlag von Blau zu Gelb vollständig erfolgt.
11. Die Extinktion bei 450 nm mit einem Mikrotiterplatten Reader **innerhalb von 15 Minuten bestimmen.**

9 BERECHNUNG DER ERGEBNISSE.

Die TSH-Konzentration der Proben wird wie folgt berechnet:

Eine Standardkurve wird erstellt, indem man die durchschnittliche Extinktion (y) der Referenzstandards gegen die entsprechende Konzentration in ng/mL (x) auf linear-linearem oder semi-log Millimeterpapier aufträgt.

Die TSH-Konzentration der Patientenprobe kann nun durch Interpolation aus dieser Standardkurve ermittelt werden. Falls vorhanden, kann die Berechnung der Daten per Computer erfolgen.

9.1 Beispielhafte Standardkurve

Die folgenden Daten dienen ausschließlich zur Demonstration und können keinesfalls zur Auswertung von Testergebnissen verwendet werden.

TSH (µIU/mL)	Optische Dichte (450 nm)
0	0,063
0,5	0,157
2	0,398
5	0,818
10	1,415
25	2,645

10 NORMALWERTE

Angaben hierzu entnehmen Sie bitte der ausführlichen englischen Arbeitsanleitung.

11 GRENZEN DES TESTS

1. Der Test muss exakt gemäß der Testanleitung des Herstellers abgearbeitet werden. Darüber hinaus muss der Benutzer sich strikt an die Regeln der GLP (Good Laboratory Practice) oder andere eventuell anzuwendende Regeln oder nationale gesetzliche Vorgaben halten.
2. Nur für den professionelle Gebrauch. Das Testergebnis allein sollte niemals als alleinige Grundlage für die Einleitung therapeutischer Konsequenzen dienen.
3. Lipämische, ikterische und/oder stark hämolysierte Proben sollten nicht verwendet werden.
4. Die Sensitivität und Präzision dieses Assays wird erheblich beeinflusst von der korrekten Durchführung des Waschschrittes!
5. Der TSH ELSIA (EIA-1782) wurde nicht mit Neugeborenen getestet und ist nicht für die Anwendung bei Neugeborenen-Screening.
6. Wenn das Testsystem nicht funktioniert, wenden Sie ein alternatives Diagnostikverfahren an oder wenden Sie sich an den Hersteller.

1 INTRODUZIONE

1.1 APPLICABILITÀ

Test enzimo-immunologico per la determinazione quanitativa dell'ormone tiroide stimolante (TSH)

1.2 importanza clinica

TSH (thyroid stimulating hormone) è una glicoproteina con un peso molecolare di ca. 28.000 Dalton e constiste in due differenti subunità alpha e beta. La determinazione di TSH o Tirotopina offre un metodo sensitivo per la diagnosi di un ipotiroidismo primario o secondario. TSH è prodotto nel lembo anteriore della ipofisi e induce la produzione e la liberazione della tiroxina (T4) e della Triiodiotyronina (T3) dalla tiroide. La secrezione del TSH reagisce sensibilmente al livello degli ormoni tiroidali T4 e T3 ed è pertanto un affidabile parametro della reale efficacia di questi ormoni nei tessuti corporei.

Anche se TSH è presente nel sangue in concentrazioni assai basse, esso è molto importante per una normale funzionalità della tiroide. Il rilascio di TSH è regolato dall'ormone TRH (TSH-releasing hormone), che è prodotto dall'ippotalamo. I livelli di TSH e TRH nel sangue sono inversamente proporzionale alla concentrazione degli ormoni tiroidali nel sangue. Se nel sangue è presente una concentrazione alta di ormone tiroidale, l'ippotalamo rilascia meno TR e di conseguenza meno TSH è prodotto e/o rilasciato. Questo meccanismo a feedback negativo serve alla regolazione del livello degli ormoni tiroidali nel sangue.

TSH e le glicoproteine pituitarie (LH, FSH, HCG) hanno in commune le stesse alpha eliche. Le catene beta differiscono, ma contengono domini con identica sequenza di aminoacidi. Quando si utilizzano antisieri TSH policlonali queste regioni omologhe possono causare indesiderate reazioni ad incrocio con le nominate glicoproteine e portare a falsi risultati in donne gravide o nel climacterium. Il test TSH ELISA utilizza un anticorpo monoclonale evitando indesiderate reazioni ad incrocio.

2 PRINCIPIO DEL TEST

Il TSH ELISA ultrasensitivo si basa sul principio del test enzimo-immunologico in fase solida. Il test usa un anticorpo monoclonale diretto contro una determinante antigenica della molecola intatta TSH. I micropozzetti sono ricoperti con L'anticorpo monoclonale murino. La soluzione del coniugato enzimatico contiene un anti-TSH anticorpo caprino, coniugato ad una perossidase del rafano. Durante l'incubazione il campione può reagire con entrambi gli anticorpi simultaneamente e le molecole TSH formeranno un complesso a sandwich con gli anticorpi. Dopo 60 minuti di incubazione a temperatura ambiente gli anticorpi non legati saranno allontanati con un passaggio di lavaggi. Un cromogeno substrato (benzidine tetrametilico, TMB/H_2O_2) è aggiunto e incubato per 20 minuti e il cromogeno vira dall'incolore al blu. L'aggiunta di 1 N HCl termina la reazione enzimatica e il colore blu vira ad un giallo chiaro.

La concentrazione di TSH è direttamente proporzionale alla intensità di colore del campione. L'estinzione è letta spettrofotometricamente a 450 nm.

3 REAGENTI

1. **Microtiter wells:** Micropozzetti, coperti con l'anticorpo monoclonale murino anti-TSH.
2. **Enzyme Conjugate**: Coniugato enzimatico in soluzione, 13 mL.
3. **Standard**: Set di referenza standard: 0, 0.5, 2, 5, 10 e 20 µlU/mL, liofillizzato (WHO, 2nd IRP, 80/558)
4. **TMB Reagent:** TMB reagente (pronto all'uso), 11 mL.
5. **Stop Solution**: Soluzione d'arresto (1N HCl), 11 mL

4 MATERIALI RICHIESTI MA NON CONTENUTI NEL KIT

- Acqua deionizzata
- Pipette a precisione: 100 µL e 1.0 mL
- Spettrofotometro per micropozzetti (450 nm lunghezza d'onda)
- Carta assorbente
- Agitatore vortex
- Materiale per il controllo qualità

5 MAGAZZINAGGIO DEI REAGENTI

Test kits non aperti dovrebbero essere magazzinati a 2 °C - 8 °C. La data di scadenza è indicata sull'etichetta del kit.

A 2 °C - 8 °C i reagenti non aperti rimangono reattivi fino alla data di scadenza indicata. Non usare reagenti oltre questa data.

Tutti i reagenti aperti devono essere magazzinati a 2 °C - 8 °C. I micropozzetti devono essere magazzinati a 2 °C – 8 °C. Una volta aperti i pacchi, questi devono essere richiusi accuratamente.

6 COLLEZIONE DEI CAMPIONI

Prelevare il sangue tramite puntura venale, lasciare coagulare e separare il siero centrifugando il campione a temperatura ambiente. Per questo test dovrebbero essere utilizzati sieri senza aggiunte.

I campioni dovrebbero essere magazzinati ben chiusi fino a 48 ore a 2 °C - 8 °C.

Campioni magazzinati per un periodo più lungo dovrebbero essere congelati solo una volta a -20 °C prima dell'analisi. Congelare soltanto una volta. Invertire campioni scongelati alcune volte prima dell'uso.

7 PREPARAZIONE DEI REAGENTI

1. Portare tutti i reagenti prima dell'uso a temperatura ambiente (18 °C - 25 °C).
2. Recostituire gli **standard liofillizzati** con **1.0 mL** acqua deionizzata. Far riposare per 20 minuti e agitare cautamente. Gli standard ricostituiti sono stabili per 30 giorni a 2 °C - 8 °C.
3. I campioni con una concentrazione di TSH superiore a 25 µIU/mL devono essere diluiti 1:10 con siero senza TSH (Standard Zero).

8 ATTUAZIONE DEL TEST

1. Posizionare il numero necessario di pozzetti nell'apposito supporto.
2. Pipettare 100 µL degli standard, dei controlli e dei campioni in ciascun pozzetto.
3. Aggiungere100 µL coniugato enzimatico in ogni pozzetto.
4. Mescolare agitando per almeno 30 secondi. Un completo mescolamento dei reagenti è molto importante!
5. Incubare per 60 minuti a temperatura ambiente (18 °C – 25 °C).
6. Vuotare i pozzetti capovolgendoli.
7. Lavare i pozzetti 5 volte con acqua deionizzata, ca. 400 µL per pozzetto.
8. Dopo l'ultimo lavaggio rimuovere le rimanenti gocce d'acqua scuotendo i pozzetti contro la carta assorbente.
9. Aggiungere 100 µL soluzione TMB in ogni pozzetto. Agitare cautamente per 10 secondi.
10. Incubare a temperatura ambiente per 20 minuti.
11. Terminare la reazione con l'aggiunta di 100 µL di Soluzione d'arresto (1N HCl).
12. Mescolare cautamente per 30 secondi. **È importante che il colore blu vira completamente al giallo.**
13. Determinare l'estinzione a 450 nm con un lettore di micropozzetti entro 15 min.

9 CALCOLO DEI RISULTATI

La concentrazione di TSH viene calcolata come segue:

Si costruisce una curva standard con le medie della estinzione in ordinata (y) degli standard e delle rispettive concentrazioni in µIU/mL sull'ascisse (x) su carta linear-lineare o semi logaritmica.

La concentrazione di TSH dei campioni può essere determinata per interpolazione con la curva standard. Il calcolo può essere esegiuto anche al calcolatore.

9.1 Curva standard esemplare

I seguenti dati servono esclusivamente per la dimostrazione e non possono essere utilizzati in alcun caso per l'analisi dei risultati.

TSH (µLU/mL)	Assorbanza (450 nm)
0	0.063
0.5	0.157
2	0.398
5	0.818
10	1.415
25	2.645

10 VALORI NORMALI

Per dettagli più precisi consultare la metodica in inglese.

11 LIMITAZIONE DEL TEST

1. Il test deve essere eseguito esattamente secondo il protocollo dato dal produttore. Inoltre l'utente deve seguire le regole del GLP (Good Laboratory Practice) o eventualmente altre regole comportamentali o disposizioni legali.
2. Solo per uso professionale. Il risultato del test da solo non è base sufficiente per lo stabilimento di una terapia.
3. Non usare campioni emolitici, itterici o lipemici.
4. Il lavaggio è un passaggio critico. Lavaggio insufficiente o la mancata rimozione d'acqua dopo i lavaggi porta a una precisione minore e a estinzioni falsamente alti.
5. Il test TSH ELISA (EIA-1782) non è stato testato sui neonati e non è destinato all'uso nello screening dei neonati.
6. Se il sistema di test non funziona, utilizzare una procedura diagnostica alternativa o contattare il produttore

SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
Σ	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité