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User's Manual

TSH rat ELISA

RUO

REF

DEV9977



96 Wells

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1 INTRODUCTION

1.1 INTENDED USE

The **DEMEDIATEC TSH rat ELISA** is an enzyme immunoassay for the quantitative measurement of TSH in rat serum. For research use only. Not for use in diagnostic procedures.

1.2 SUMMARY AND EXPLANATION

Thyroid stimulating hormone (also known as thyrotropin or TSH) is a glycoprotein produced by the anterior pituitary gland. Through its action on the thyroid gland, it plays a major role in maintaining normal circulating levels of the iodothyronines, T4 and T3. The production and secretion of TSH is controlled on the one side by negative feedback from circulating T4 and T3, and on the other side by the hypothalamic thyrotropin-releasing hormone (TRH).

The TSH molecule is composed of two non-identical subunits, α and β , that are bound together in a noncovalent manner. Within a species, the TSH α unit is structurally identical to the α subunits of related glycoprotein hormones (LH, FSH). The β subunits of the related hormones are structurally hormone-specific and therefore determine their unique biological activities.

The mechanism controlling thyroid function in rats is exactly analogous to the mechanism operating in humans. This means that thyrotropin-releasing hormone stimulates the release of TSH from the pituitary gland as well as the serum concentrations of T4 and T3 influence the action of the pituitary gland.

This similarity between rat and human thyroid physiology makes the rat a very useful model for evaluating the effects of new drugs on thyrometabolic status.

2 PRINCIPLE

The test kit is a solid phase enzyme immunometric assay (ELISA) in the microplate format, designed for the quantitative measurement of TSH in rat serum. The microplate is coated with a monoclonal antibody specific for TSH.

Calibrators and samples are pipetted into the antibody coated microplate. Afterwards, a polyclonal horseradish peroxidase-labeled antibody is added. During a 18-20 hours incubation at 4°C sandwich complexes consisting of the two antibodies and the rat TSH is formed. Non-reactive components are removed by a washing step.

A chromogenic substrate, TMB (3,3',5,5'-Tetra-Methyl-Benzidine), is added to all wells. During a 30 minutes incubation, the substrate is converted to a colored end product (blue) by the fixed enzyme. Enzyme reaction is stopped by dispensing of hydrochloric acid as stop solution (change from blue to yellow). The color intensity is direct proportional to the concentration of rat TSH present in the sample.

The optical density of the color solution is measured with a microplate reader at 450 nm. Bi-chromatic measurement with a 600 - 690 nm reference filter is recommended.

3 WARNINGS AND PRECAUTIONS

1. For professional use only.
2. Before starting the assay, read the instructions completely and carefully. Use the valid version of the package insert provided with the kit. Be sure that everything is understood.
3. Do not mix reagents of different lots. Do not use expired reagents.
4. The microplate contains snap-off strips. Unused wells must be stored at 2 - 8°C in the sealed foil pouch and used in the frame provided.
5. Avoid contact with Stop Solution. It may cause skin irritation and burns.
6. Pipetting of samples and reagents must be performed as quickly as possible and in the same sequence for each step.
7. Change pipette tips between samples and reagents to avoid carry over contamination.

8. 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.
9. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
10. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
11. Assay reagents contain Thimerosal against microbial growth. In case of contact with eyes or skin, flush immediately with water.
12. All reagents should be at room temperature (21-26°C) before use. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
13. TMB substrate has an irritant effect on skin and mucosa. In case of contact with skin or eyes, wash thoroughly with water. Please note that extreme temperature changes may cause spontaneous decay of the peroxide.

4 REAGENTS

4.1 REAGENTS PROVIDED

1. **Microtiterplate**, 12 x 8 (break apart) strips with 96 wells, ready to use; Wells coated with a monoclonal TSH antibody.
2. **Rat TSH Master Calibrator**, 1 vial, 80 ng, lyophilized, in buffer matrix containing highly purified rat TSH, **For reconstitution see "Reagent preparation"**.
3. **Enzyme-Labeled anti rat TSH Antibody**, 1 vial, 22 ml, red, ready to use; contains a horseradish peroxidase-labeled polyclonal anti TSH antibody (goat), in a buffered solution with preservative.
4. **TMB-Substrate Solution**, 1 vial, 22 ml each, ready to use; contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.
5. **Stop Solution**, 1 vial, 7 ml, ready to use; contains 2 M Hydrochloric Acid solution.
6. **Wash Buffer Concentrate**, 1 vial, 50 ml (10X concentrated); **see „Reagent preparation“**.
7. **Rat TSH Calibrator/Sample Diluent**, 1 vial, 6 ml, ready to use.
8. **Adhesive Cover**

4.2 MATERIALS REQUIRED BUT NOT PROVIDED

- Centrifuge
- A microtiter plate reader capable for endpoint measurement at 450 nm
- Vortex mixer
- Calibrated variable precision micropipettes (25 µl, 50 µl, 100 µl, 200 µl and 1000 µl).
- Test tubes for preparation of standard solution series
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

4.3 REAGENT PREPARATION

All reagents should be at room temperature before use.

Calibrators:

Reconstitute lyophilized Rat TSH Master Calibrator with **1 ml dest. water** 30 min. before use (end concentration of 80 ng/ml). Make a dilution series with Calibrator/Sample Diluent to get calibrators with 80, 40, 20, 10, 5 and 2.5 ng/ml.

Wash Solution:

Dilute 50 ml of 10X concentrated *Wash Solution* with 450 ml deionized water to a final volume of 500 ml. *The diluted Wash Solution is stable for at least 3 months at room temperature.*

4.4 STORAGE CONDITIONS

When stored at 2°C to 8°C all reagents are stable until expiration date or 30 days after opening.

The Stop Solution is stable up to 2 months after opening or until the expiration date.

The Wash Solution is stable for 3 months after dilution or until the expiration date.

Store Calibrators refrigerated, they will be stable at 2°C to 8°C for 7 days after reconstitution or until expiration date. For longer storage freeze at -20°C.

Protect Divisible Microplate from moisture. Store together with desiccant and carefully sealed in the plastic bag.

Protect TMB-Substrate Solution from light.

5 SPECIMEN

For determination of rat TSH serum is the preferred sample matrix. The procedure calls for 25 µl matrix per well. The samples may be stored refrigerated at 2 – 8 °C for one week, or up to 2 months frozen at -20 °C. To avoid repeated thawing and freezing the samples should be aliquoted.

Samples expected to contain rat TSH concentrations higher than the highest calibrator (80 ng/ml) should be diluted with the zero calibrator before assay. The additional dilution step has to be taken into account for the calculation of the results.

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 and 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.
- For internal quality control we suggest to use **Rat Control Set coded DEV99RC. For more information please contact DEMEDITEC.**

6.2 ASSAY PROCEDURE

Each run must include a standard curve.

1. Prepare a sufficient number of microplate wells to accommodate calibrators and samples in duplicates.
2. Preparation of calibrators. Label five tubes: F (40 ng/ml), E (20 ng/ml), D (10 ng/ml), C (5 ng/ml) and B (2.5 ng/ml). Pipet **0.1 ml** of the Calibrator/Sample Diluent into all tubes. Pipet 0.1 ml of the reconstituted Rat TSH Master Calibrator into tube F (40 ng/ml), and mix thoroughly. Repeat this process successively to complete the 2-fold dilution series. The reconstituted Rat TSH Calibrator will serve as the highest calibrator G (80 ng/ml). Use the Rat TSH Calibrator/Sample Diluent as the zero Calibrator A

	1	2	3	4	5	6	7	8	9	10	11	12
a	A	E	P2	P..								
b	A	E	P2	P..								
c	B	F	P3									
d	B	F	P3									
e	C	G	P4									
f	C	G	P4									
g	D	P1	P5									
h	D	P1	P5									

2. Pipet **25 µl** of each **calibrator, control** and **sample** into the wells prepared.
3. Add **200 µl** of **Enzyme-Labeled Anti-Rat TSH antibody** to all wells.
4. Incubate for **18-20 hours at 4°C**.
5. Discard the content of the wells and wash **4 times** with **300 µl buffered wash solution**.
Remove as much wash solution as possible by beating the microplate carefully.
6. Add **200 µl** of **TMB/Substrate Solution** to all wells.
7. Incubate for **30 minutes** in the dark.
8. Add **50 µl** of **Stop Solution** to each well and mix carefully.
9. Read the optical density at **450 nm**. Bi-chromatic measurement with a reference at 600 - 690 nm is recommended.

The developed color is stable for at least 15 minutes. Read optical densities during this time.

6.3 CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of calibrators, 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 calibration curve.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log are recommended.
5. The concentration of the samples can be determined directly from this calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted. For the calculation of the concentrations, this dilution factor has to be taken into account.

6.3.1 Example of Typical Calibrator Curve

Following data are intended for illustration only and should not be used to calculate results from another run.

Standard	Absorbance Units
Calibrator A (0 ng/ml)	0.075
Calibrator B (2.5 ng/ml)	0.191
Calibrator C (5 ng/ml)	0.283
Calibrator D (10 ng/ml)	0.514
Calibrator E (20 ng/ml)	0.983
Calibrator F (40 ng/ml)	1.935
Calibrator G (80 ng/ml)	3.657

7 EXPECTED NORMAL VALUES

In order to determine the normal range of serum TSH in rat, samples of male and female rats were collected and analyzed using the DEMEDITEC TSH rat ELISA kit. The following ranges are calculated with the results of this study.

Rat	Sex	N	Range (ng/ml)
Wistar	Female	49	0.85 – 3.23
Sprague-Dawley	Female	6	0.85 – 2.38
Sprague-Dawley	Male	6	2.44 – 9.14

It is recommended that each laboratory establish its own normal range since TSH levels can vary due to handling and sampling techniques.

8 PERFORMANCE CHARACTERISTICS

8.1 ANALYTICAL SENSITIVITY

The lowest analytical detectable level of TSH that can be distinguished from the Zero Calibrator is 0.1 ng/ml at the 2SD confidence limit.

8.2 SPECIFICITY

The following materials have been evaluated for cross reactivity at 10 ng/ml. The percentage indicates cross reactivity at 50% displacement compared to TSH.

Steroid	% Cross reaction
Rat LH	n.d.
Rat FSH	n.d.

n.d. = non detectable

8.3 REPRODUCIBILITY

8.3.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of 6 serum samples within one run. The within-assay variability is shown below:

Mean (ng/ml)	12.38	26.04	40.55	1.28	2.51	6.77
SD	0.37	0.97	1.52	0.16	0.22	0.40
CV (%)	3.0%	3.7%	3.8%	12.4%	8.8%	5.9%
n =	20	20	20	20	20	20

8.3.2 Inter-Assay

The inter-assay (between-run) variation of 3 serum samples was determined in 9 different assays.

Mean (ng/ml)	9.47	20.92	34.28
SD	0.79	1.34	1,82
CV (%)	8.3	6.4	5.3
n =	9	9	9

8.4 RECOVERY

Using the Calibrator Matrix three spiking solutions were prepared (A = 560 ng/mL, B = 280 ng/mL and C = 140 ng/mL). A 50 µL aliquot of each solution was spiked into 950 µL of three different sera, for a spiking ratio of 1 to 19, leaving the serum matrix of the spiked samples relatively intact. All samples were then measured by rat TSH procedure. To calculate expected values 95% of the unspiked values were added to 5% of the spiking solution concentrations.

Serum	Spiking Solution	Observed (O)	Expected (E)	O/E %
4	-	0.9	-	-
	A	25.8	28.9	89%
	B	11.9	14.9	80%
	C	6.3	7.9	80%
5	-	0.9	-	-
	A	27.3	28.9	94%
	B	12.1	14.9	81%
	C	6.4	7.9	81%
6	-	1.0	-	-
	A	23.9	29.0	82%
	B	11.1	15.0	74%
	C	6.2	8.0	78%

8.5 LINEARITY

Three serum samples were assayed undiluted and diluted with the calibrator matrix.

Serum	Dilution	Observed (O)	Expected (E)	O/E %
1	native	24.39	-	-
	1 in 2	13.46	12.20	110.3%
	1 in 4	6.41	6.10	105.1%
	1 in 8	3.34	3.05	109.5%
2	native	14.86	-	-
	1 in 2	8.48	7.43	114.1%
	1 in 4	3.90	3.72	104.8%
	1 in 8	2.12	1.86	114.0%
3	native	35.68	-	-
	1 in 2	19.35	17.84	108.5%
	1 in 4	9.64	8.92	108.1%
	1 in 8	4.90	4.46	109.9%











9 LIMITATIONS OF PROCEDURE



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.

9.1 DRUG INTERFERENCES

Until now no substances (drugs) are known influencing the measurement of rat or mouse TSH in serum. Lipemic and haemolysed samples can cause false results.

SYMBOLS USED WITH DEMEDITEC ELISA

Symbol	English	Deutsch	Francais	Espanol	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consultez le Mode d'emploi	Consulte las Instrucciones	Consulti le istruzioni
	In vitro diagnostic device	In-vitro-Diagnostikum	Diagnostic in vitro	Diagnóstico in vitro	Diagnostica in vitro
	Catalogue number	Katalog-Nr.	Référence	No de catálogo	No. di Cat.
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeitsdatum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
<i>Distributed by</i>	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
<i>Content</i>	Content	Inhalt	Contenu	Contenido	Contenuto
<i>Volume/No.</i>	Volume / No.	Volumen/Anzahl	Volume/Numéro	Volumen/Número	Volume/Quantità
<i>Microtiterwells</i>	Microtiterwells	Mikrotiterwells	Plaques de microtitration	Pocillos de la Microplaca	Micropozzetti
<i>Antiserum</i>	Antiserum	Antiserum	Antisérum	Antisero	Antisiero
<i>Enzyme Conjugate</i>	Enzyme Conjugate	Enzymkonjugat	Conjugué enzymatique	Conjugado enzimático	Tracciante enzimatico
<i>Enzyme Complex</i>	Enzyme Complex	Enzymkomplex	Complexe enzymatique	Complex enzimático	Complesso enzimatico
<i>Substrate Solution</i>	Substrate Solution	Substratlösung	Solution substrat	Solución de sustrato	Soluzione di substrato
<i>Stop Solution</i>	Stop Solution	Stopplösung	Solution d'arrêt	Solución de paro	Soluzione d'arresto
<i>Zero Standard</i>	Zero Standard	Nullstandard	Standard 0	Standard 0	Standard zero
<i>Standard</i>	Standard	Standard	Standard	Calibrador	Standard
<i>Control</i>	Control	Kontrolle	Contrôle	Control	Controllo
<i>Assay Buffer</i>	Assay Buffer	Assaypuffer	Tampon d'essai	Tampón de ensayo	Tampone del test
<i>Wash Solution</i>	Wash Solution	Waschlösung	Solution de lavage	Solución de lavado	Soluzione di lavaggio
<i>1N NaOH</i>	1N NaOH	1N NaOH	1N NaOH	1N NaOH	1N NaOH (idrossido di sodio 1N)
<i>1 N HCl</i>	1 N HCl	1 N HCl	1N HCl	1 N HCl	
<i>Sample Diluent</i>	Sample Diluent	Probenverdünnungsmedium	Solution pour dilution de l'échantillon		Diluyente dei campioni
<i>Conjugate Diluent</i>	Conjugate Diluent	Konjugatverdünnungsmedium	Solution pour dilution du conjugué		Diluyente del tracciante

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Conformidade com as normas europeias	Europæisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Instruções de uso	Brugermanual	Användar manual	Εγχειρίδιο χρήστη
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
RUO				
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevaringstemperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
<i>Distributed by</i>				
<i>Content</i>	Conteúdo	Indhold	Innehåll	Περιεχόμενο
<i>Volume/No.</i>	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..
<i>Microtiterwells</i>	Alvéolos de microtitulação	Mikrotiterbrønde	Brunnar i Mikrotiterplatta	Πηγαδάκια Μικροτιτλοδοτήσεως
<i>Antiserum</i>	Anti-soro	Antiserum	Antiserum	Αντιορός
<i>Enzyme Conjugate</i>	Conjugado enzimático	Enzymkonjugat	Enzymkonjugat	Συζευγμένο ενζυμο
<i>Enzyme Complex</i>	Complexo enzimático	Enzymkompleks	Enzymkomplex	Σύμπλοκο ενζύμου
<i>Substrate Solution</i>	Solução de substrato	Substratopløsning	Substratlösning	Διάλυμα υποστρώματος
<i>Stop Solution</i>	Solução de paragem	Stopopløsning	Stopp lösning	Διάλυμα τερματισμού
<i>Zero Standard</i>	Padrão zero	Standard 0	Standard 0	Πρότυπο Μηδέν
<i>Standard</i>	Calibrador	Standard	Standard	Πρότυπα
<i>Control</i>	Controlo	Kontrol	Kontroll	Έλεγχος
<i>Assay Buffer</i>	Tampão de teste	Assay buffer	Assay Buffer	Ρυθμιστικό Διάλυμα Εξέτασης
<i>Wash Solution</i>	Solução de lavagem	Vaskebuffer	Tvätt lösning	Διάλυμα πλύσεως
<i>1N NaOH</i>	1N NaOH	1N NaOH	1N NaOH	1N NaOH
<i>1 N HCl</i>	1 N HCl	1 N HCl	1 N HCl	1 N HCl
<i>Sample Diluent</i>				
<i>Conjugate Diluent</i>				