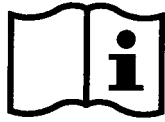


Product information

Information about other products is available at: www.demeditec.com



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-linked immunosorbent 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 16-24 hours incubation at 4-8°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.

3 WARNINGS AND PRECAUTIONS

1. This kit is for research 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. 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.
4. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
5. 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.
6. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
7. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
8. Allow the reagents to reach room temperature (18-25°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
9. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
10. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
11. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
12. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
13. Do not use reagents beyond expiry date as shown on the kit labels.
14. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.
15. 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.
16. Avoid contact with Stop Solution. It may cause skin irritation and burns.
17. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
18. For information please refer to Material Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from Demeditec Diagnostics GmbH.

4 REAGENTS

4.1 REAGENTS PROVIDED

1. **SORB | MT** **Microtiter Plate**, 12 x 8 (break apart) strips with 96 wells, ready to use; Wells coated with a monoclonal anti-rat TSH antibody.
2. **CAL** **Master Calibrator**, 1 vial, 80 ng, lyophilized, in buffer matrix containing highly purified rat TSH, **For reconstitution see "Reagent preparation"**.
3. **ENZ | CONJ** **Enzyme Conjugate**, 1 vial, 22 ml, red, ready to use; contains a horseradish peroxidase-labeled polyclonal anti TSH antibody in a buffered solution with preservative.
4. **SUB | TMB** **Substrate Solution**, 1 vial, 22 ml each, ready to use; contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.
5. **STOP | SOLN** **Stop Solution**, 1 vial, 7 ml, ready to use; contains 2 N Hydrochloric Acid solution.
6. **WASH | SOLN | 10x** **Wash Solution**, 1 vial, 50 ml (10X concentrated); see „Reagent preparation“.
7. **SAM | DIL** **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 12 weeks at room temperature.*

4.4 STORAGE CONDITIONS

When stored at 2-8°C unopened reagents will be stable until expiration date. Do not use reagents beyond this date. Opened reagents must be stored at 2-8°C. After first opening the reagents are stable for 30 days if used and stored properly.

Microtiter wells must be stored at 2-8°C. Take care that the foil bag is sealed tightly.

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

4.5 DISPOSAL OF THE KITS

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

4.6 DAMAGED TEST KITS

In case of any severe damage of the test kit or components, DEMEDITEC have to be informed written, 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 SAMPLES

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 6 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 Calibrator/Sample Diluent 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, controls 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 Master Calibrator into tube F (40 ng/ml), and mix thoroughly. Repeat this process successively to complete the 2-fold dilution series. The reconstituted Calibrator will serve as the highest calibrator G (80 ng/ml). Use the 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 Conjugate** to all wells.
4. Mix for 10 seconds and incubate for **16-24 hours at 4-8°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 **Substrate Solution** to all wells.
7. Incubate for **30 minutes** at room temperature in the dark.
8. Add **50 µl** of **Stop Solution** to each well and mix carefully.
9. Read the optical density at **450 nm**.
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. Construct a standard curve by plotting the mean absorbance obtained from each calibrator 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 IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred calculation method. 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 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 0.081 ng/ml at the 2SD confidence limit.

8.2 SPECIFICITY

Chemically similar substances were tested for their cross reactivity to the specific analyte.

Steroid	Cross reaction
Rat LH	1.6 – 2.8%
Rat FSH	0.3%

8.3 REPRODUCIBILITY

8.3.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of 3 samples within one run. The within-assay variability is shown below:

	Sample 1	Sample 2	Sample 3
Mean (ng/ml)	4.47	8.76	15.32
SD (ng/ml)	0.15	0.16	0.52
CV (%)	3.4	1.8	3.4
n =	20	20	20

8.3.2 Inter-Assay

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

	Sample 1	Sample 2	Sample 3
Mean (ng/ml)	4.35	8.40	14.48
SD (ng/ml)	0.38	0.39	0.91
CV (%)	8.8	4.6	6.3
n =	9	9	9

8.4 LINEARITY

Three serum samples were assayed undiluted and diluted with the calibrator matrix. The percentage linearity was calculated by comparing the expected and measured values.

Serum	Dilution	Measured Concentration (ng/ml)	Expected Concentration (ng/ml)	Linearity %
1	native	30.12	-	-
	1 in 2	17.92	15.06	119%
	1 in 4	9.17	7.53	122%
	1 in 8	4.20	3.77	111%
2	native	23.00	-	-
	1 in 2	13.81	11.50	120%
	1 in 4	6.79	5.75	118%
	1 in 8	3.43	2.88	119%
3	native	15.39	-	-
	1 in 2	8.63	7.70	112%
	1 in 4	4.70	3.85	122%
	1 in 8	2.36	1.92	123%








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 TSH in serum. Lipemic and haemolysed samples can cause false results.

SYMBOLS USED WITH DEMEDITEC ELISA

Symbol	English	Deutsch	Français	Espanol	Italiano
	European Conformity	CE-Konformitätskennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	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