

# Product information

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# TSH rat ELISA

RUO

**REF** DEV9977

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## 1 INTRODUCTION

### 1.1 Intended use

The **DemeditecTSH rat ELISA** is an enzyme immunoassay for the quantitative measurement of TSH in rat serum and EDTA plasma. 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,  $\alpha$  and  $\beta$ , that are bound together in a noncovalent manner. Within a species, the TSH  $\alpha$  subunit is structurally identical to the  $\alpha$  subunits of related glycoprotein hormones (LH, FSH). The  $\beta$  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 2-8°C sandwich complexes consisting of the two antibodies and the rat TSH are formed. Non-reactive components are removed by a washing step.

A chromogenic substrate, TMB (3,3',5,5'-tetramethylbenzidine), is added to all wells. During a 30 minutes incubation, the substrate is converted to a colored end product (blue) by the bound enzyme. Enzyme reaction is stopped by dispensing hydrochloric acid as stop solution (change from blue to yellow). The color intensity is directly proportional to the concentration of rat TSH present in the sample. The Optical Density (OD) of the color solution is measured with a microplate reader at 450 nm.

## 3 WARNINGS AND PRECAUTIONS

1. This kit is intended for research only. Use by staff, who is specially informed and trained in methods which are carried out by use of immunoassays.
2. All blood components and biological materials should be handled as potentially hazardous in use and for disposal. Follow universal precautions when handling and disposing of infectious agents.
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 2-8°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 washing steps.
9. Allow the reagents to reach room temperature (18-25°C) before starting the test. Temperature will affect the Optical Density readings of the assay.
10. Never pipet by mouth and avoid contact of reagents and samples with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where samples or kit reagents are handled.
12. Wear disposable 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 microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different microplates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the microplates may result slightly different.
17. Avoid contact with Stop Solution. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, CMIT and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
20. For information please refer to Safety Data Sheets available upon request directly from Demeditec Diagnostics GmbH or on the Demeditec homepage ([www.demeditec.com](http://www.demeditec.com)).
21. If product information, including labeling, is incorrect or inaccurate, please contact the kit manufacturer or supplier.

## 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 LYQ Master Calibrator**, 1 vial, 80 ng, lyophilized, in buffered matrix containing highly purified rat TSH; for reconstitution see "4.4 Reagent preparation".
3. **ENZ CONJ 100x Enzyme Conjugate**, 1 vial, 0.3 ml (100x concentrated), red; contains a horseradish peroxidase-labeled polyclonal anti-rat TSH antibody in a buffered solution with preservative; for dilution see "4.4 Reagent preparation".
4. **ENZ CONJ DIL Enzyme Conjugate Diluent**, 1 vial, 30 ml, red; ready to use.
5. **SUB TMB Substrate Solution**, 1 vial, 22 ml each, ready to use; contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.
6. **STOP SOLN Stop Solution**, 1 vial, 7 ml, ready to use; contains 2 N hydrochloric acid solution. Avoid contact with the stop solution. It may cause skin irritations and burns.
7. **WASH SOLN 10x Wash Solution**, 1 vial, 50 ml (10x concentrated); for dilution see "4.4 Reagent preparation".
8. **SAM DIL Calibrator/Sample Diluent**, 1 vial, 6 ml, ready to use.
9. **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 Calibrator solution series
- Absorbent paper
- Distilled or deionized water
- Timer
- Semi logarithmic graph paper or software for data reduction

### 4.3 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 at 2-8°C for seven days or at < -20°C (in aliquots) for 30 days after reconstitution.

#### 4.4 Reagent preparation

Allow the reagents and the required number of wells to reach room temperature (18-25°C) before starting the test.

##### Calibrators:

Reconstitute lyophilized rat TSH Master Calibrator **CAL** **LYO** with 1 ml dist. water 30 min. before use (final concentration 80 ng/ml). Prepare a dilution series with **Calibrator/Sample Diluent** **SAM** **DIL** to get calibrators with 80, 40, 20, 10, 5, and 2.5 ng/ml.

##### Wash Solution:

Dilute 50 ml of 10x concentrated **Wash Solution** **WASH** **SOLN** **10x** with 450 ml deionized water to a final volume of 500 ml. The diluted 1x Wash Solution is stable for at least 12 weeks at room temperature (18-25°C). Precipitates may form when stored at 2-8°C, which should dissolve again by swirling at room temperature (18-25°C). The Wash Solution should only be used when the precipitates have completely dissolved.

##### Enzyme Conjugate:

Immediately before use dilute Enzyme Conjugate Concentrate **ENZ** **CONJ** **100x** 1:100 in Enzyme Conjugate Diluent **ENZ** **CONJ** **DIL**, for example 0.1 ml Enzyme Conjugate Concentrate **ENZ** **CONJ** **100x** + 9.9 ml Enzyme Conjugate Diluent **ENZ** **CONJ** **DIL**. Mix thoroughly.

**Attention: Please prepare only immediately before use!**

#### 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 Safety Data Sheet.

#### 4.6 Damaged test kits

In case of any severe damage of the test kit or components, Demeditec has 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 and EDTA plasma are the preferred sample matrices. The procedure calls for 25 µl sample 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** **SAM** **DIL** 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 samples must be allowed to come to room temperature (18-25°C) 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 Calibrator, 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.
- Respect the incubation times as stated in this instructions for use.
- Microplate washing is important. Improperly washed wells will give erroneous results. It is recommended to use a multichannel pipette or a multistepper, respectively, or an automatic microplate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during washing and aspiration. Wash and fill all reagents with care. While washing, check that all wells are filled precisely with Wash Solution, and that no residues remain in the wells.
- Duplicate determination of Calibrators, Controls, and Samples is recommended in order to identify potential pipetting errors.
- A Calibrator curve must be established for every run.
- For internal quality control we suggest to use **Rat Control Set coded DEV99RC**. For more information please contact Demeditec.

### 6.2 Assay Procedure

1. Prepare a sufficient number of microtiter plate 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. Successively repeat this process 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									

3. Dispense **25 µl** of each **Calibrator, Control and Sample with new disposable tips** into appropriate wells.
4. Immediately before use dilute **Enzyme Conjugate Concentrate** **ENZ** **CONJ** **100x** 1:100 in **Enzyme Conjugate Diluent** **ENZ** **CONJ** **DIL** (see chapter 4.4). Add **200 µl** of **diluted Enzyme Conjugate** to all wells.
5. Mix for 10 seconds and incubate for **16-24 hours at 2-8°C**.
6. Discard the content of the wells and wash **4 times** with **300 µl diluted 1x Wash Solution** Remove as much Wash Solution as possible by beating the microplate carefully.
7. Add **200 µl** of **Substrate Solution** **SUB** **TMB** to all wells.
8. Incubate for **30 minutes** at room temperature (18-25°C) in the dark.
9. Add **50 µl** of **Stop Solution** **STOP** **SOLN** to each well and mix carefully.
10. Determine the Optical Density (OD) of each well at 450 nm. It is recommended to read the wells within 15 minutes.

### 6.3 Calculation of results

1. Calculate the average Optical Density (OD) values for each set of Calibrators, Controls, and samples.
2. The obtained optical densities of the Calibrators (y-axis, linear) are plotted against their corresponding concentrations (x-axis, logarithmic) either on semi-logarithmic paper or using an automated method.
3. Using the mean OD value for each sample determine the corresponding concentration from the Calibrator curve.
4. Automated method: The results in this Instruction for Use 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 different results.
5. The concentration of the samples can be read directly from this Calibrator curve. Samples with concentrations higher than that of the highest calibrator have to be further diluted and reassayed. 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 must not be used to calculate results from another run.

Calibrator	OD at 450 nm
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 and EDTA plasma 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.

Population	Gender	n	Range (ng/ml)	Mean (ng/ml)	Median (ng/ml)	2.5 - 97.5 percentile (ng/ml)
Rat Serum	male	12	n.d. - 4.67	1.28	0.81	n.d. - 4.44
Rat Serum	female	11	0.69 - 3.49	1.66	1.63	0.79 - 3.02
Rat EDTA Plasma	male	10	n.d. - 3.98	1.52	0.89	n.d. - 3.95
Rat EDTA Plasma	female	5	0.54 - 2.96	1.25	0.85	0.55 - 2.79

n.d.: not detectable

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

## 8 QUALITY CONTROL

Good laboratory practice requires that controls are run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The use of control samples is advised to assure the day-to-day validity of results. For internal quality control we suggest to use **Rat Control Set coded DEV99RC**.

Employ appropriate statistical methods for analyzing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices, microplate reader, 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 Demeditec directly.

## 9 PERFORMANCE CHARACTERISTICS

### 9.1 Analytical sensitivity

The lowest analytical detectable level of rat TSH that can be distinguished from the Zero Calibrator is 0.081 ng/ml at the 2SD confidence limit.

### 9.2 Specificity

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

Analyte	Cross-reaction
Rat LH	1.6 - 2.8%
Rat FSH	0.3%

### 9.3 Reproducibility

#### 9.3.1 Intra-Assay

The intra-assay variation was determined by 20 replicate measurements of three samples within one run using the Demeditec TSH rat ELISA.

	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

#### 9.3.2 Inter-Assay

The inter-assay variation was determined by duplicate measurements of three samples in nine different runs using the Demeditec TSH rat ELISA.

	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

### 9.4 Linearity

Three samples were assayed undiluted and diluted with Zero Calibrator. The percentage linearity was calculated by comparing the expected and measured concentrations.

Sample	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%



## 10 LIMITATIONS OF PROCEDURE

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

### 10.1 Interferences

- Until now no substances (drugs) are known influencing the measurement of rat TSH in serum or EDTA-plasma.
- Lipemic, icteric, and hemolyzed samples can cause false results.
- Samples containing sodium azide should not be used in the assay.
- Non-specific interferences with this in vitro immunoassay cannot be excluded. If unplausible results are suspected, they should be considered invalid and verified by further testing.

## 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 a sufficient number of controls within the test procedure for validating the accuracy and precision of the test.

The test results are valid only if all controls meet 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 Demeditec Diagnostics GmbH.

### 11.2 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.

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 REVISION HISTORY OF INSTRUCTION FOR USE

Changes compared to the previous version 4-02/22 to the current version 5-08/24.

Section 1.1 & 5 Rat EDTA-Plasma can also be used in this assay

Section 4 & 6.2 Enzyme Conjugate is provided as a 100x concentrated solution and needs to be diluted immediately before use.

Section 7 expected values updated

General editorial changes


Changes compared to the previous version 5-08/24 to the current version 5a-08/24.

Section 4.1 **Enzyme Conjugate Diluent**, 1 vial, 30 ml, red; ready to use.





## SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Française	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	utilisation 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 catalogo
	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 y advertencias precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Température 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>	Distributed by	Vertrieb durch	Distribution par	Distribución por	Distribuzione da parte di
	Version	Version	Version	Versión	Versione
	Single-use	Einmalverwendung	À usage unique	Uso único	Uso una volta