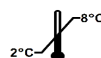
Thyrotropin (TSH) (Rat) ELISA Kit

BGT-KET-228

96Wells

REF BGT-KET-228



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For research use only – Not for use in diagnostic procedures

Thyrotropin (TSH) (Rat) ELISA Kit

1. INTRODUCTION

1.1 INTENDED USE

The **Thyrotropin (TSH) (Rat) ELISA Kit** 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. Standards 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 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 package insert 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 rinsing steps.
9. Allow the reagents to reach room temperature (18 – 25 °C) before starting the test. Temperature will affect the absorbance 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 latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiterplate readers.

16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with Stop Solution. 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. Safety Data Sheets for this product are available upon request directly from the manufacturer.
21. If product information, including labeling, is incorrect or inaccurate, please contact the kit manufacturer or supplier.

4. REAGENTS

4.1 REAGENTS PROVIDED

	96	**Microtiter Plate** – Ready to use
Contents:	12 x 8 (break apart) strips with 96 wells; Wells coated with a monoclonal anti-rat TSH antibody.	

	STANDARD	**Standard** – Lyophilized
Contents:	In buffer matrix containing highly purified rat TSH.	
Volume:	1 x 80 ng	

For reconstitution see "Reagent preparation".

	CONJUGATE	**Conjugate** – Ready to use
Contents:	Contains a horseradish peroxidase-labeled polyclonal anti TSH antibody in a buffered solution with preservative.	
Volume:	1 x 22 ml, red	

	SUBSTRATE	**Substrate Solution** – Ready to use
Contents:	Contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix.	
Volume:	1 x 22 ml	

	STOP-SOLN	**Stop Solution** – Ready to use
Contents:	Contains 2 N Hydrochloric Acid solution.	
Volume:	1 x 7 ml	
Hazards identification:	 H290 May be corrosive to metals. H314 Causes severe skin burns and eye damage. H335 May cause respiratory irritation.	

	WASH-CONC 10x	**Wash Solution** – 10X concentrated
Volume:	1 x 50 ml	

See „Reagent preparation".

	DILUENT	**Diluent** – Ready to use
Volume:	1 x 6 ml	

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.

Standards:
Reconstitute lyophilized Rat TSH Standard with **1 ml dest. water** 30 min. before use (end concentration of 80 ng/ml). Make a dilution series with **Diluent** to get standards 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 (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.

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 Standard 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 Safety Data Sheet.

4.6 DAMAGED TEST KITS

In case of any severe damage of the test kit or components, the manufacturer 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 serum 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 standard (80 ng/ml) should be diluted with the **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 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 standard, control or sample in order to avoid cross contamination.
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- As a general rule the enzymatic reaction is linearly proportional to time and temperature.
- Respect the incubation times as stated in this instructions for use.
- Microtiter plate 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 microtiter

plate washing system. Do not allow the wells to dry between incubations. Do not scratch coated wells during rinsing and aspiration. Rinse and fill all reagents with care. While rinsing, check that all wells are filled precisely with Wash Solution, and that there are no residues in the wells.

- Duplicate determination of standards, controls and samples is recommended in order to identify potential pipetting errors.
- A standard curve must be established for every run.
- For internal quality control we suggest to use **Rat Control Set. For more information please contact the manufacturer.**

6.2 ASSAY PROCEDURE

1.	Prepare a sufficient number of microplate wells to accommodate standards, controls and samples in duplicates.
2.	Preparation of standards. 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 Diluent into all tubes. Pipet 0.1 ml of the reconstituted Standard into tube F (40 ng/ml), and mix thoroughly. Repeat this process successively to complete the 2-fold dilution series. The reconstituted Standard will serve as the highest Standard G (80 ng/ml). Use the Diluent as the Standard 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.	Pipet **25 µl** of each **standard, control** and **sample** into the wells prepared.
4.	Add **200 µl** of **Enzyme Conjugate** to all wells.
5.	Mix for 10 seconds and incubate for **16 – 24 hours at 4 – 8 °C**.
6.	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.
7.	Add **200 µl** of **Substrate Solution** to all wells.
8.	Incubate for **30 minutes** at room temperature in the dark.
9.	Add **50 µl** of **Stop Solution** to each well and mix carefully.
10.	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 standards, controls and patient samples.
2. The obtained optical densities of the standards (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 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 standard 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 Standard Curve

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

Standard	Absorbance Units
Standard A (0 ng/ml)	0.075
Standard B (2.5 ng/ml)	0.191
Standard C (5 ng/ml)	0.283
Standard D (10 ng/ml)	0.514
Standard E (20 ng/ml)	0.983
Standard F (40 ng/ml)	1.935
Standard 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 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. QUALITY CONTROL

Good laboratory practice requires that controls are run with each standard curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance. The use of control samples is advised to assure the day-to-day validity of results. Use controls at both normal and pathological levels.
For internal quality control we suggest to use **Rat Control Set.**
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices, microtiter plate 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 the manufacturer directly.

9. PERFORMANCE CHARACTERISTICS

9.1 ANALYTICAL SENSITIVITY

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

9.2 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%

9.3 REPRODUCIBILITY

9.3.1 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

9.3.2 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

9.4 LINEARITY

Three serum samples were assayed undiluted and diluted with the standard 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%

10. 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.

10.1 INTERFERENCES

- Until now no substances (drugs) are known influencing the measurement of rat TSH in serum.
- Lipemic, icteric and haemolysed 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 the manufacturer.

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.