



User's Manual

Rat TSH ELISA Kit



DEIASL209



96T



This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

 Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

 Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe)  Fax: 1-631-938-8221

 Email: info@creative-diagnostics.com  Web: www.creative-diagnostics.com

PRODUCT INFORMATION

Intended Use

The 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.

General Description

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.

Principles of Testing

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.

Reagents And Materials Provided

1. Microtiter Plate: 12x8 (break apart) strips with 96 wells; Wells coated with an anti-Rat TSH antibody. Ready to use.
2. Standards: highly purified Rat TSH in serum. Lyophilized, 1 x 80 ng.
3. Enzyme Conjugate: contains a horseradish peroxidase-labeled polyclonal anti TSH antibody, in a buffered solution with preservative, 1 x 22 ml. Ready to use.

4. TMB Substrate Solution: contains tetramethylbenzidine (TMB) and hydrogen peroxide in a buffered matrix, 1 x 22 ml. Ready to use.
5. Stop Solution: contains 2 N Hydrochloric Acid solution, 1 x 7 ml. Ready to use.
6. Wash Solution Concentrate (10x): 1 x 50 ml
7. Diluent: 1 x 6 ml. Ready to use.
8. Adhesive Cover

Materials Required But Not Supplied

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

Storage

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

Specimen Collection And Preparation

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 Standard (80 ng/ml) should be diluted with the Standard/Sample Diluent before assay. The additional dilution step has to be taken into account for the calculation of the results.

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 Standard/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.

Assay Procedure

Before Assay Notes:

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard and sample in order to avoid cross contamination.
4. 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.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.
6. For internal quality control we suggest to use Rat Control Set. For more information please contact the manufacturer.
7. Each run must include a standard curve.

Assay Steps:

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Preparation of standard. 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 Standard/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.

Calculation

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. 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 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. For the calculation of the concentrations this dilution factor has to be taken into account.

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

Reference 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.

Precision

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

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

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.

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 %

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 %

Precautions

1. The kit is strictly intended for veterinary use 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 result in colored solution. 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. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink, or apply cosmetics in areas where specimens 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 procedure 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 vary slightly.
17. Avoid contact with Stop Solution. It may cause skin irritation and burns.
18. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline and regulation.
19. For information please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from the manufacturer.

Limitations

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 GLP (Good Laboratory Practice). Any improper handling of samples or modification of this test might influence the results.

