Rat TSH ELISA Kit

Enzyme Immunoassay for the quantification of Thyroid stimulating hormone (TSH) in rat serum.

Catalog number: ARG80664

Package: 96 wells

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INTRODUCTION

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 alpha 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.

PRINCIPLE OF THE ASSAY

This assay employs the sandwich quantitative enzyme immunoassay technique. An antibody specific for rat TSH has to be bound onto a pre-coated microtiter plate. Standards or samples are pipetted into the wells and any rat TSH present is bound by the immobilized antibody. Then a Horseradish Peroxidase (HRP)-conjugated antibody for rat TSH is added to each well and incubate. Following a washing to remove unbound substances, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of rat TSH bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450 nm ±2 nm. The concentration of rat TSH in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 strips x 8-well	4°C.
Rat TSH Master Calibrator	1 vial (80 ng)	4°C, lyophilized
HRP conjugated antibody	22 ml (ready for use)	4°C
Rat TSH Calibrator/Sample Diluent	6 ml (ready for use)	4°C
10X Wash Buffer	50 ml	4°C
TMB substrate	22 ml (ready for use)	4°C (Protect from light)
STOP solution	7 ml (ready for use)	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
- Use reservoirs only for single reagents, especially applies to the substrate reservoirs. The substrate solution might be colored when using a reservoir which has been used for the HRP conjugated antibody before.
- Do not pour reagents back into vials as reagent contamination may occur.
- Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Allow the reagents to reach room temperature (21-26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the samples will not be affected.
- If crystals are observed in the 10X Wash buffer, warm to RT until the

crystals are completely dissolved.

- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- Samples contain azide cannot be assayed.

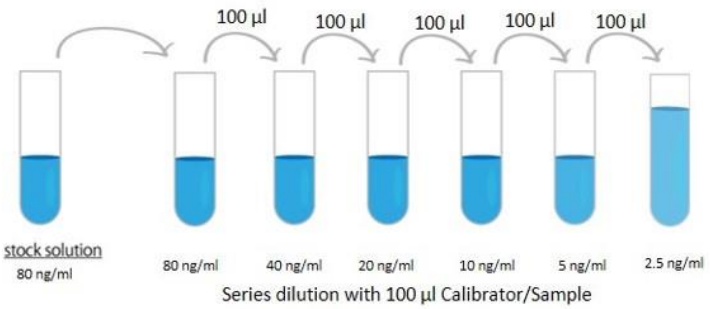
SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer. The diluted Wash buffer is stable for 12 weeks at room temperature.
- Sample: If the initial assay found samples contain TSH higher than the highest standard, or the sample expected to contain TSH higher than the highest standard, the samples can be diluted with Calibrator/Sample Diluent and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.
- Calibrators: Reconstitute lyophilized Rat TSH Master Calibrator with 1 ml distilled water 30 min before use (end concentration of 80 ng/ml). Store reconstituted standard at 2°C- 8°C for 7 days. Aliquot and store at-20°C for long term storage. Make a dilution series with Calibrator/Sample Diluent to get calibrators with 80, 40, 20, 10, 5 and 2.5 ng/ml and the Calibrator /Sample diluent buffer serves as zero standard (0 ng/ml).



Series dilution with 100 µl Calibrator/Sample

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **25 µl** of **standards and samples** in duplicate into sample wells.
3. Add **200 µl** of **HRP conjugated antibody** into each well. Mix for 10 seconds and incubate for **16-24 hours at 4°C**.
4. Aspirate each well and wash, repeating the process 3 times for a **total 4 washes.** Wash by filling each well with **1X wash buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
5. Add **200 µl** of **TMB substrate** to each well. Incubate for **30 minutes at room temperature** in dark.
6. Add **50 µl** of **Stop Solution** to each well.
7. Read the OD with a microplate reader at **450 nm** immediately. The developed color is stable for at least 15 min, it is recommended read the samples within 15 min after adding stop solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-log 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 standard curve.

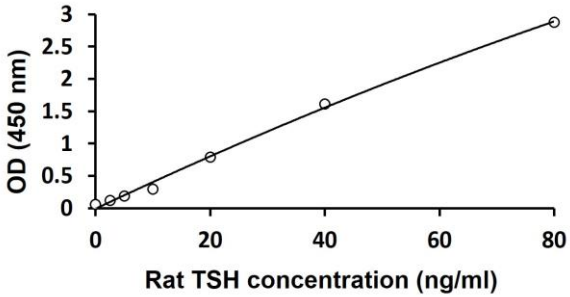
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

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.

Specificity

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

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 2.8% and inter-assay precision was 6.5%.

Linearity

111-123%