Rat TSH ELISA Kit

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

Catalog number: ARG80664
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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.

<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
<th>Storage information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody-coated microplate</td>
<td>12 strips x 8-well</td>
<td>4°C.</td>
</tr>
<tr>
<td>Rat TSH Master Calibrator</td>
<td>1 vial</td>
<td>4°C, lyophilized</td>
</tr>
<tr>
<td>HRP conjugated antibody</td>
<td>22 ml (ready for use)</td>
<td>4°C</td>
</tr>
<tr>
<td>Rat TSH Calibrator/Sample Diluent</td>
<td>6 ml (ready for use)</td>
<td>4°C</td>
</tr>
<tr>
<td>10X Wash Buffer</td>
<td>50 ml (ready for use)</td>
<td>4°C</td>
</tr>
<tr>
<td>TMB substrate</td>
<td>22 ml (ready for use)</td>
<td>4°C (Protect from light)</td>
</tr>
<tr>
<td>STOP solution</td>
<td>7 ml (ready for use)</td>
<td>4°C</td>
</tr>
</tbody>
</table>
MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm (optional: read at 610-650 nm as the reference wave length)
- 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.
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- If crystals are observed in the 10X Wash buffer, warm to RT (not more than 50°C) 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 2 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **1X Wash buffer**: Dilute 10X Wash buffer into distilled water to yield 1X Wash buffer.
- **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
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distilled water 30 min before use (end concentration of 80 ng/ml). Store reconstituted standard at 2°C - 8°C for 7 days. 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).

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. Incubate for 18-20 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
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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. (optional: read at 600-690 nm as the reference wave length)

CALCULATION OF RESULTS

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

2. Using linear 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. 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.

![TSH standard curve graph]

QUALITY ASSURANCE

Sensitivity
The lowest analytical detectable level of TSH that can be distinguished from the Zero Calibrator is 0.1 ng/ml at the 2SD confidence limit.

Specificity
The following materials have been evaluated for cross reactivity at 10 ng/ml. The percentage indicates cross reactivity at 50% displacement compared to TSH.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>% Cross reaction</th>
</tr>
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<tbody>
<tr>
<td>Rat LH</td>
<td>Non detectable</td>
</tr>
<tr>
<td>Rat FSH</td>
<td>Non detectable</td>
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Intra-assay and Inter-assay precision
The CV value of intra-assay precision was 6.27% and inter-assay precision was 6.67%.

Recovery
74-94%

Linearity
104.8-114.1%