

# RODENT TSH ELISA TEST LYOPHILIZED STANDARDS

The Rodent TSH ELISA test is an immunoassay designed for the quantitative determination of thyroid stimulating hormone (TSH) in serum/plasma samples of rat/mouse and related species.

## TEST PRINCIPLE:

The Rodent TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes affinity purified antibody directed against intact rodent TSH molecule for solid phase (microtiter wells) immobilization and a mouse anti-TSH antibody is in the antibody-enzyme (horseradish peroxidase) conjugate. The test sample is allowed to react simultaneously with the two antibodies, resulting in the TSH molecules being sandwiched between the solid phase and enzyme-linked antibodies. After 3 hours of incubation period at 37 °C, the wells are washed with wash solution to remove unbound-labeled antibodies. A solution of TMB is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of stop solution and the color is changed to yellow and measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the color intensity of the test sample.

## REAGENTS AND MATERIALS PROVIDED:

1. Antibody-coated microtiter wells
2. Reference standard Lyophilized set (0, 1, 2.5, 5, 10, 25 ng/mL). QC1(2-4ng/mL), QC2(10-15ng/mL), Reconstitute in one mL with standard/sample diluent.
3. Enzyme Conjugate, 12mL
4. TMB color reagent, 12mL
5. 20X Wash buffer, 20 mL
6. Stop solution (2N HCl), 6mL
7. Standard/Sample Diluent, 20 mL

## MATERIALS REQUIRED, BUT NOT PROVIDED

1. Precision pipettes: 50uL, 100uL, 200uL, and 1.0mL
2. Disposable pipette tips
3. Vortex mixer or equivalent
4. Absorbent paper or paper towel
5. Graph paper
6. Microtiter plate reader

## SPECIMEN COLLECTION AND PREPARATION

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum/plasma samples only.

## STORAGE OF TEST KIT AND INSTRUMENTATION

**Note of Caution: Immediately after receiving the kit unopened test kits should be stored at 4-8°C. The microtiter plate should always be kept in a sealed bag with desiccants to minimize exposure to damp air at room temperature. Opened test kits will remain stable until the expiration date shown, provided it is stored as prescribed above. Do not leave any reagents at room temperature more than 3 hours.**

A microtiter plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 OD or greater at a 450nm wavelength is acceptable for use in absorbency measurement.

## REAGENT PREPARATION

1. All reagents should be brought to room temperature (25-28°C) before use.
2. Lyophilized standards should be diluted with one ml of standard/sample diluent.
3. Dilute wash buffer, desired amount with distilled water (1 part with 19 parts). The buffer is stable for 1-3 months, if stored at 4-8°C.

## ASSAY PROCEDURE



**One must follow accurately these steps to ensure correct results. Technician must make sure all the lyophilized standards dissolved completely before the assay. Use clean pipettes and sterile, disposable tips:**

1. Secure the desired number of coated wells in the holder.
2. Dispense 100ul of standards, specimens, and controls into appropriate wells.
3. Dispense 100ul of Enzyme Conjugate Reagent into each well.
4. Thoroughly shake the plate for 30 seconds. It is very important to shake the plate very well at this step.
5. Incubate at 37C for 3 hours in a sealed container or use zip-lock bag (provided).
6. Remove the incubation mixture by decanting the plate contents into a waste container.
7. Rinse and decant the microtiter wells five (5) times with wash buffer.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. Dispense 100ul of TMB solution into each well. Gently mix for 10 seconds.
10. Incubate at room temperature for 20 minutes.
11. Stop reaction by adding 50ul (one drop) of stop solution, 2N HCl to each well.
12. Gently mix for 30 seconds. It is important to observe a color change from blue to yellow.
13. Read optical density at 450nm with a microtiter well reader.

*Important note:* The wash step is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

#### **CALCULATION OF RESULTS**

Calculate the mean absorbency value (A450) for each set of reference standards, specimens, controls and test samples. Construct a standard curve by plotting the mean absorbency obtained from each reference standard against its concentration in ng/ml on graph paper, with absorbency values on the vertical or Y axis, and concentration on the horizontal or X axis. Use the mean absorbency values for each specimen to determine the corresponding concentration of TSH in ng/ml from the standard curve.

#### **EXPECTED VALUES AND SENSITIVITY**

The minimal detectable concentration of Rodent TSH by this assay is estimated to be 0.2 ng/ml. and the normal and experimental values should be established in your own laboratory. Each lab must follow good lab practice and maintain proper documentation.

#### **Limitations and Warranty**

The present rodent TSH ELISA is designed for helping the scientist to analyze test samples from rodent species only. There are no warranties, expressed, implied or otherwise indicated, which extend beyond this description of this product. Endocrine Technologies, Inc. is not liable for property or laboratory damage, personal injury, or test samples loss, or economic loss caused by this product. Warranty is limited to replacement of similar ELISA Kit or reagents damaged during shipment or leaking solutions or any other issues within 30 days, with written explanation, with sharing the entire data including raw printouts from the reader and return of the ELISA product. The analyst should establish the standard curve and a small number of samples before proceeding to analyze a large number of samples. Endocrine will not replace or refund, only if deemed necessary on scientific merits within 30 days only.

#### **REFERENCES**

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**Rodent TSH ELISA TEST KIT                      Product Profile & Information**  
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