Human Thyroid Stimulating Hormone (TSH) ELISA Kit

Catalog No: abx153404

Size: 96T

Range: 0.3 mIU/L - 12 mIU/L

Sensitivity: < 0.14 mIU/L

Storage: Store at 2-8°C for 6 months.

Application: For quantitative detection of TSH in Human Serum.

Principle of the Assay: This kit is based on sandwich enzyme-linked immuno-sorbent assay technology. An antibody specific to TSH is pre-coated onto a 96- well plate. The standards and samples are added to the wells and incubated. HRP conjugated anti-TSH antibody is used as detection antibody. Next, TMB substrate solution is added only wells that contain TSH, HRP-conjugated antibody will produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of the color yellow is proportional to the TSH amount bound on the plate. The O.D. absorbance is measured spectrophotometrically at 450 nm in a microplate reader, and then the concentration of TSH can be calculated.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Wash Buffer (20X): 15 ml
- Standard: 6 x 0.5 ml
- Detection Reagent: 6 ml
- Stop Solution: 6 ml
- TMB substrate: 9 ml
- Plate Sealer: 4

Materials Required But Not Provided

- 37°C incubator
- Microplate reader (wavelength: 450 nm)
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- ELISA shaker
- Deionized or distilled water
- Tubes to prepare standard or sample dilutions
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- 0.01 mol/L PBS (pH 7.0 - 7.2)

Protocol

A. Sample Preparation

Isolate the test samples soon after collecting, analyze immediately or store at 4°C for up to 5 days. Otherwise, store at -20°C for up to one month or -80°C for up to two months to avoid loss of bioactivity. Avoid multiple freeze-thaw cycles.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 2 hr. Centrifuge at approximately 1000 × g for 20 mins. If a precipitate appears, centrifuge again. Assay immediately or aliquot and store at -20°C or -80°C.

Notes:

- Please bring sample slowly to room temperature. Sample hemolysis will influence the result. Hemolyzed specimen should not be used.
- Samples must be diluted so that the expected concentration falls within the kit's range. Sample should be diluted in 0.01 mol/L PBS (pH 7.0 - 7.2).
- If the sample are not indicated in the manual's applications, a preliminary experiment to determine the validity of the kit will be necessary.
- Fresh sample or recently obtained samples are recommended to prevent protein degradation and denaturalization that may lead to erroneous results.
- Always use non-pyrogenic, endotoxin-free tubes for blood collection.

B. Reagent Preparation

Wash Buffer: Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 15 ml of concentrated wash buffer into 285 ml of distilled water).

Standard: The standard is provided in 6 tubes with concentrations: 12 mIU/L, 6 mIU/L, 3 mIU/L, 1.5 mIU/L, 0.6 mIU/L and 0.3 mIU/L.

C. Assay Protocol

Equilibrate the kit components and samples to room temperature before use. It is recommended to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and then, record their positions. It is recommended to measure each standard and sample in duplicate. Add the solution at the bottom of each well without touching the side walls.
2. Add 100 µl of each standard solution into the standard wells.
3. Add 100 µl of 0.01 mol/L PBS (pH 7.0 - 7.2) into the control (zero) wells.
4. Add 100 µl of appropriately diluted sample into the test sample wells. Shake the plate mildly to mix thoroughly.
5. Aliquot 50 µl of the Detection Reagent to each well. Seal the plate with a cover and incubate for 2 h at 37°C.
6. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. Fill each well completely with 1X Wash Buffer (350 µl) using a multi-channel Pipette or auto washer (1-2 minute soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.
7. Aliquot 90 µl of TMB Substrate into each well. Seal the plate with a cover and incubate at 37°C for 10-20 min. Avoid exposure to light. The incubation time is for reference use only, the optimal time should be determined by end user. Do not exceed 30 min.
8. Add 50 µl of Stop solution into each well. There should be a color change to yellow. Gently tap the plate to ensure thorough mixing.

9. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately.

For calculation, average the O.D.450 duplicate readings for each reference standard, control and each sample and subtract the average control (zero) O.D.450 reading. The standard curve can be plotted as the relative O.D.450 of each reference standard solution (Y) vs. the respective concentration of each standard solution (X). The TSH concentration of the samples can be interpolated from the standard curve.

Note: If the samples measured were diluted, multiply the dilution factor by the interpolated concentration of the sample to obtain the concentration before dilution.

D. Precautions

1. Before using the kit, centrifuge the tubes briefly to bring down the contents trapped in the lid.
2. Wash buffer may crystallize and separate. If this happens warm to room temperature and mix gently until the crystals are completely dissolved.
3. Avoid foaming or bubbles when mixing or reconstituting components. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
4. Do not let the wells uncovered for extended periods between incubation. Once reagents are added to the wells, avoid letting the strips dry as this can inactivate the biological material on the plate. Incubation time and temperature must be controlled.
5. Ensure plates are properly sealed or covered during incubation steps.
6. Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings.
7. Do not reuse pipette tips and tubes to avoid cross contamination.
8. The TMB Substrate solution is easily contaminated; work under sterile conditions when handling the TMB substrate solution. The TMB Substrate solution should also be protected from light. Unreacted substrate should be colorless or very light yellow in appearance. Aspirate the dosage needed with sterilized tips and do not dump the residual solution back into the vial.

E. Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of TSH were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of TSH were tested on 3 different plates, 8 replicates in each plate.

CV (%) = (Standard Deviation / mean) × 100

Inter-Assay: CV<12%

Intra-Assay: CV<10%