



Product Manual

TSH (human) ELISA kit

Catalog #: ENZ-KIT131

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Please read entire booklet before proceeding with the assay.



Carefully note the handling and storage conditions of each kit component.



Please contact Enzo Life Sciences Technical Support if necessary.

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INTENDED USE

The TSH (human) ELISA Kit is intended for the determination of TSH in human serum. For research use only, not for diagnostic procedures.

SUMMARY AND EXPLANATION

Thyroid Stimulating Hormone (TSH) is a glycoprotein hormone secreted by the pituitary gland and regulates the synthesis and release of T3 and T4 by the thyroid gland. TSH has two subunits, alpha and beta. The alpha subunit is similar to the alpha subunit found in LH, FSH and hCG glycoprotein hormones. However, the beta subunit is specific and differs from hormone to hormone. The beta subunit is responsible for its biological activity. The serum TSH measurement is one of the most important tools in the diagnosis of thyroid disorders. Increased serum TSH is an early and sensitive indicator of decreased thyroid reserve and overt primary hypothyroidism. A decreased TSH level is an indicator of TSH-independent hyperthyroidism (Graves disease). The sensitivity of this ELISA test is 0.05 μ IU/ml.

PRINCIPLE OF THE TEST

The TSH is a solid phase sandwich ELISA method. The samples and anti-TSH-HRP/Biotin conjugate are added to the wells coated with Streptavidin. TSH in the sample forms a sandwich between two specific antibodies to TSH. Unbound protein and HRP conjugate are washed off by the wash buffer. Upon the addition of the substrate, the intensity of color is proportional to the concentration of TSH in the samples. A standard curve is prepared relating color intensity to the concentration of the TSH.

| MATERIALS PROVIDED | 96 Tests |
|---|-----------------|
| 1. Microwells coated with Streptavidin | 12x8x1 |
| 2. TSH Standard: 7 vials (ready to use) | 0.5ml |
| 3. TSH Conjugate Reagent: 1 bottle (ready to use) | 12ml |
| 4. TMB Substrate: 1 bottle (ready to use) | 12ml |
| 5. Stop Solution: 1 bottle (ready to use) | 12ml |
| 6. 20X Wash concentrate: 1 bottle | 25ml |

SAFETY WARNINGS & PRECAUTIONS**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

Handle with
care

- Potential biohazardous materials: The standards set contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
- This test kit is designed for research use only.
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.
- Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.



Reagents
require
separate
storage
conditions.

STORAGE

1. Store the kit at 2-8°C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until the expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light.

OTHER MATERIALS NEEDED

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450nm
5. Absorbance paper or paper towel
6. Graph paper

SPECIMEN COLLECTION HANDLING

1. Collect blood specimens and separate the serum immediately.
2. Specimens may be stored refrigerated at (2-8°C) for 5 days. If storage time exceeds 5 days, store frozen at (-20°C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.
5. Do not use grossly lipemic specimens.

REAGENT PREPARATION

Prepare 1X Wash buffer by adding the contents of the bottle (25ml, 20X) to 475ml of distilled or deionized water. Store at room temperature (18-26°C).

ASSAY PROCEDURE

Prior to assay, allow reagents to stand at room temperature (18-26°C). Gently mix all reagents before use.

1. Place the desired number of coated strips into the holder.
2. Pipet 50µl of TSH standards, control and samples into the designated wells.
3. Add 100µl of ready-to-use conjugate reagent to all wells. Shake for 10 to 30 seconds.
4. Cover the plate and incubate for 60 minutes at room temperature (18-26°C).
5. Remove liquid from all wells. Wash wells three times with 300µl of 1X wash buffer. Blot on absorbent paper towel.
6. Add 100µl of TMB substrate to all wells.
7. Incubate for 15 minutes at room temperature.
8. Add 50µl of stop solution to all wells. Shake the plate gently to mix the solution.
9. Read absorbance on ELISA Reader at 450nm within 15 minutes after adding the stop solution.

CALCULATION OF RESULTS

The standard curve is constructed as follows:

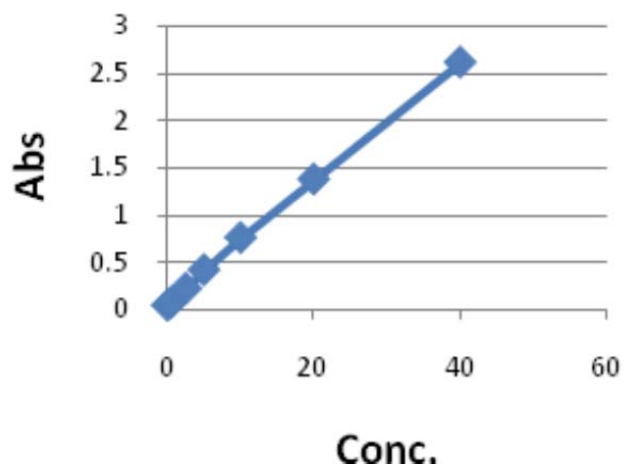
1. Check TSH standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the TSH standards (vertical axis) versus the TSH standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

TYPICAL RESULTS

The results shown below are for illustration only and should not be used to calculate results.

| | OD 450nm | Conc. μ IU/mL |
|-------|----------|-------------------|
| Std 1 | 0.033 | 0 |
| Std 2 | 0.062 | 0.5 |
| Std 3 | 0.21 | 2.5 |
| Std 4 | 0.41 | 5 |
| Std 5 | 0.75 | 10 |
| Std 6 | 1.37 | 20 |
| Std 7 | 2.61 | 40 |

Standard Curve



EXPECTED VALUES

It is recommended that each laboratory establish its own normal ranges based on a representative sampling of the local population. The following values for TSH may be used as initial guideline ranges only:

| Classification | Normal Range (μ IU/ml) |
|--------------------|-----------------------------|
| Adults | 0.4-4.2 |
| Newborn (1-4 days) | 1.0-39 |
| 2-20 weeks | 1.7-9.0 |
| 21 weeks-20 years | 0.7-6.4 |

LIMITATIONS OF THE TEST

Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

REFERENCES

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