Product Manual

Human Thyroid- Stimulating Hormone (TSH) ELISA Kit

Catalog Numbers

MET- 5051	96 assays
MET- 5051- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

Thyroid-stimulating hormone (TSH) is a glycoprotein hormone secreted by the pituitary gland. TSH stimulates the thyroid gland to produce two hormones: triiodothyronine (T_3) and thyroxine (T_4); these key hormones regulate metabolism in most tissues of the body. Serum TSH measurement is one of the most important tools in the diagnosis of thyroid disorders. Elevated serum TSH, combined with low T_3/T_4 levels, is a sensitive indicator of decreased thyroid reserve and overt primary hypothyroidism. Decreased TSH level is a indicator of TSH-independent hyperthyroidism (Graves' disease). Ultimately, TSH measurement has become an essential screening and monitoring tool for many thyroid issues.

Cell Biolabs' TSH ELISA Kit is an enzyme immunoassay developed for detection and quantitation of the human TSH protein. The kit has a detection sensitivity limit of 20 pg/mL TSH. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and TSH samples.

Assay Principle

An anti-TSH coating antibody is adsorbed onto a microtiter plate. TSH protein present in the sample or standard binds to the antibodies adsorbed on the plate; a biotinylated anti-TSH antibody is added and binds to the antigen captured by the first antibody. Following incubation and wash steps, a streptavidin-enzyme conjugate is added and binds to the biotinylated anti-TSH antibody. Unbound streptavidin-enzyme conjugate is removed during a wash step, and substrate solution is added to the wells. A colored product is formed in proportion to the amount of TSH present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from purified TSH and sample concentration is then determined.

Related Products

1. MET-5052: Human Adiponectin ELISA Kit
2. PRB-5044: Human Alpha 1 Antitrypsin ELISA Kit
3. PRB-5047: Human CK-MB ELISA Kit
4. PRB-5050: Human Cardiac Troponin I ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-TSH Antibody Coated Plate (Part No. 50511B): One strip well 96-well plate.
2. Biotinylated Anti-TSH Antibody (1000X) (Part No. 50512D): One 20 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human TSH Standard (Part No. 50513D): One 100 µL vial of 1 µg/mL human TSH.

Materials Not Supplied

1. TSH Sample: serum, plasma, cell or tissue lysate
2. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
3. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receiving, aliquot and store TSH Standard at -20ºC and avoid freeze/thaw. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-TSH Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-TSH Antibody 1:1000 and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

1. Prepare a dilution series of TSH Standard in the concentration range of 1 ng/mL – 0.016 ng/mL by diluting the stock solution in Assay Diluent (Table 1).

Standard Tubes	1 µg/mL Human TSH Standard (µL)	Assay Diluent (µL)	TSH (ng/mL)
1	4	3996	1
2	500 of Tube #1	500	0.5
3	500 of Tube #2	500	0.25
4	500 of Tube #3	500	0.125
5	500 of Tube #4	500	0.063
6	500 of Tube #5	500	0.031
7	500 of Tube #6	500	0.016
8	0	500	0

Table 1. Preparation of TSH Standard

Assay Protocol

1. Prepare and mix all reagents thoroughly before use.
2. Add 100 µL of TSH sample or standard to the Anti-TSH Antibody Coated Plate. Each TSH sample, standard, blank, and control should be assayed in duplicate.
3. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
4. Remove plate cover and empty wells. Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Biotinylated Anti-TSH Antibody to each well.
6. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
7. Remove plate cover and empty wells. Wash the strip wells 5 times according to step 4 above.
8. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well.
9. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.
10. Remove plate cover and empty wells. Wash microwell strips 5 times according to step 4 above. Proceed immediately to the next step.
11. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

    Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
12. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
13. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical TSH ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

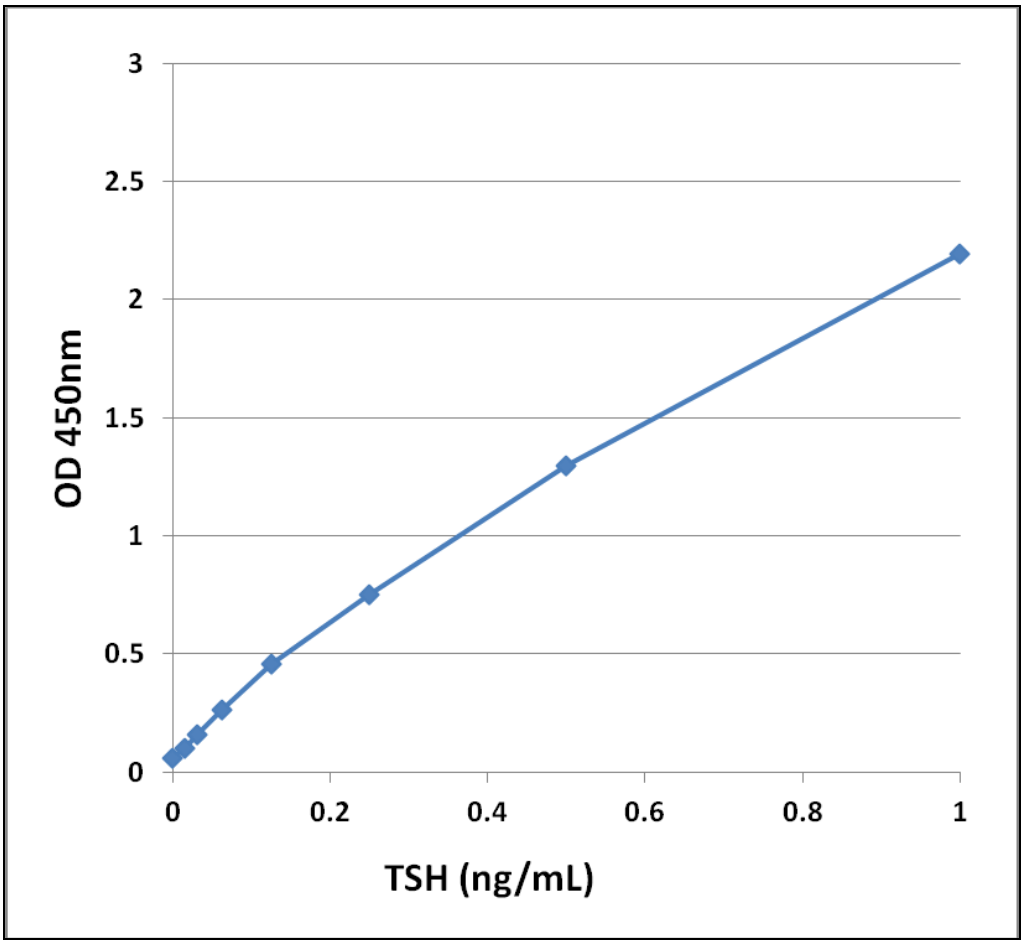

Figure 1: TSH ELISA Standard Curve

References

1. Frank, J., J. Faix, R. Hermos, D. Mullaney, D. Rojan, M. Mitchell, R. Klein (1996) *J. Pediatr.* ***128****:548-554.*
2. Maes, M., K. Mommen, D. Hendrickx, D. Peeters, P. D'Hondt, R. Ranjan, F. De Meyer, S. Scharp'e (1997) *Clin. Endocrinol.* **46**:587-598.
3. Morimoto, K., K. Inouye (1997) *J. Immunol. Methods* **205**:81-90.

Warranty

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com