EAGLE BIOSCIENCES

Thyroid Stimulating Hormone (TSH) ELISA Assay Kit

Catalog Number:
THH31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0 (12 DEC 23)

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INTENDED USE

The Eagle Biosciences Thyroid Stimulating Hormone (TSH) ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the direct quantitative determination of thyroid stimulating hormone in human serum. The Eagle Biosciences Thyroid Stimulating Hormone (TSH) ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Thyroid stimulating hormone (TSH) is a glycoprotein hormone secreted by the anterior pituitary gland. TSH has two subunits, namely α and β. The α subunit of TSH is similar to the α subunit found in the LH, FSH and hCG glycoprotein hormones. The β subunit however, is specific and differs from hormone to hormone. The thyroid hormones are secreted and produced by the thyroid gland. The production of thyroid hormones is under the regulation of TSH. Also, TSH acts as a stimulator of iodide transport and the gland itself is under the positive control of TSH. The concentrations of thyroid hormones control the secretion of TSH, therefore, a negative feedback exists. It is to be noted that the secretion of thyroid hormones are under the direct, positive effect of the sympathetic nervous system. The major protein component of the thyroid gland is thyroglobulin, a glycoprotein of which the secretion in the blood stream is stimulated by TSH. Therefore, TSH plays an important role in the proper function and development of the thyroid gland. It is recommended to assay both the glycoprotein hormone and the target organ hormones. For example, in primary hypothyroidism the serum level of thyroxine is low while the TSH level is high. In secondary hypothyroidism, both thyroxine and TSH are low. The TSH level is decreased in hyperthyroidism. Today, with all the sensitive assays available, if there were to be only one test to be prescribed for thyroid function, TSH would be the test. TSH determinations are also helpful to monitor patients who receive thyroxine replacement therapy.

PRINCIPLE OF THE ASSAY

The principle of the following enzyme immunoassay test follows a typical one-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for TSH is immobilized onto the microplate and another monoclonal antibody specific for a different region of TSH is conjugated to horse radish peroxidase (HRP). TSH from the sample and standards are allowed to bind simultaneously to the plate and to the HRP conjugate. The washing and decanting steps remove any unbound HRP conjugate. After the washing step, the enzyme substrate is added. The enzymatic reaction is terminated by addition of the stopping solution. The absorbance is measured on a microtiter plate reader. The intensity of the colour formed by the enzymatic reaction is directly proportional to the concentration of TSH in the sample. A set of standards is used to plot a standard curve from which the amount of TSH in patient samples and controls can be directly read.

PROCEDURAL CAUTIONS AND WARNINGS

1. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
2. Control materials or serum pools should be included in every run at a high and low level for assessing the reliability of results.
3. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
4. In order to reduce exposure to potentially harmful substances, gloves should be worn when handling kit reagents and human specimens.
5. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.

6. A calibrator curve must be established for every run.
7. The controls should be included in every run and fall within established confidence limits.
8. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
10. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
11. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
12. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
13. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
14. Kit reagents must be regarded as hazardous waste and disposed of according to national regulations.

LIMITATIONS

1. All the reagents within the kit are calibrated for the direct determination of TSH in human serum. The kit is not calibrated for the determination of TSH in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only calibrator A may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. This kit is for research use only and should not be used for diagnostic procedures.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the standards and controls has been tested and found to be nonreactive for Hepatitis B surface antigen and has also been tested for the presence of antibodies to HCV and Human Immunodeficiency Virus (HIV) and found to be negative. No test method however, can offer complete assurance that HIV, HCV and Hepatitis B virus or any infectious agents are absent. The reagents should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen.

CHEMICAL HAZARDS

Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

SPECIMEN COLLECTION AND STORAGE

Approximately 0.2 mL of serum is required per duplicate determination. Collect 4–5 mL of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

SPECIMEN PRETREATMENT

This assay is a direct system; no specimen pretreatment is necessary.

REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Precision pipettes to dispense 50, 100, 150 and 300 µL
2. Disposable pipette tips
3. Distilled or deionized water
4. Plate shaker
5. Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10)

REAGENTS PROVIDED

1. **Mouse Anti-TSH Antibody-Coated Break-Apart Well Microplate** — Ready To Use
   - Contents: One 96-well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

2. **Mouse Anti-TSH Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate** — Requires Preparation X50
   - Contents: Anti-TSH monoclonal antibody-HRP conjugate in a protein-based buffer with a non-mercury preservative.
   - Volume: 300 µL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µL of HRP in 2 mL of assay buffer). If the whole plate is to be used dilute 240 µL of HRP in 12 mL of assay buffer. Discard any that is left over.

3. **TSH Calibrators** — Ready To Use
   - Contents: Six vials containing TSH in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of TSH. Calibrated against World Health Organization (WHO) 2nd IS 80/558.

   * Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator A	0 µIU/mL	2.0 mL
Calibrator B	0.2 µIU/mL	0.5 mL
Calibrator C	1 µIU/mL	0.5 mL
Calibrator D	5 µIU/mL	0.5 mL
Calibrator E	15 µIU/mL	0.5 mL
Calibrator F	30 µIU/mL	0.5 mL

   - Storage: Refrigerate at 2–8°C.

Stability: 12 months in unopened vials or as indicated on label. Once opened, the standards should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

4. **Controls** — Ready To Use
   - Contents: Two vials containing TSH in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of TSH. Refer to vial labels for the acceptable range.
   - Volume: 0.5 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months in unopened vial or as indicated on label. Once opened, the controls should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

5. **Wash Buffer Concentrate** – Requires Preparation x10
   - Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
   - Volume: 50 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.
   - Preparation: Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 mL of the wash buffer concentrate in 450 mL of water.

6. **Assay Buffer** – Ready to Use
   - Contents: One bottle containing a protein-based buffer with a non-mercury preservative.
   - Volume: 15 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

7. **TMB Substrate** – Ready to Use
   - Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
   - Volume: 16 mL/vial
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

8. **Stopping Solution** – Ready to Use
   - Contents: One bottle containing 1M sulfuric acid.
   - Volume: 6 mL/bottle
   - Storage: Refrigerate at 2–8°C
   - Stability: 12 months or as indicated on label.

ASSAY PROCEDURE

Specimen Pretreatment: None.

All reagents must reach room temperature before use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. Prepare working solutions of the anti-TSH-HRP conjugate and wash buffer.
2. Remove the required number of well strips. Reseal the bag and return any unused strips to the refrigerator.
3. Pipette 50 µL of each calibrator, control and specimen sample into correspondingly labelled wells in duplicate.
4. Pipette 100 µL of the conjugate working solution into each well. (We recommend using a multichannel pipette.)
5. Incubate on a plate shaker (approximately 200 rpm) for 90 minutes at room temperature.
6. Wash the wells 3 times with 300 µL of diluted wash buffer per well and tap the plate firmly against absorbent paper to ensure that it is dry (The use of a washer is recommended).
7. Pipette 150 µL of TMB substrate into each well at timed intervals.
8. Incubate on a plate shaker for 10–15 minutes at room temperature (or until calibrator F attains dark blue colour for desired OD).
9. Pipette 50 µL of stopping solution into each well at the same timed intervals as in step 7.
10. Read the plate on a microplate reader at 450 nm within 20 minutes after addition of the stopping solution.

* If the OD exceeds the upper limit of detection or if a 450 nm filter is unavailable, a 405 or 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

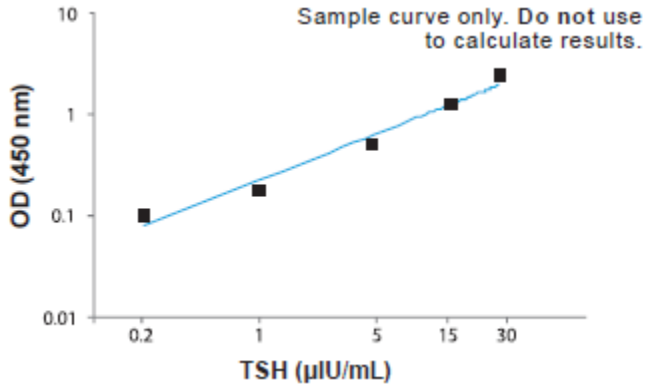
1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the "0" calibrator from the mean absorbance values of the calibrators, controls and serum samples.
4. Draw a calibrator curve on log-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
5. Read the values of the unknowns directly off the calibrator curve.
6. If a sample reads more than 30 µIU/mL, then dilute it with calibrator A at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	OD 1	OD 2	Mean OD	Value (µIU/mL)
A	0.071	0.073	0.072	0
B	0.100	0.099	0.100	0.2
C	0.177	0.171	0.174	1
D	0.492	0.527	0.510	5
E	1.270	1.254	1.262	15
F	2.391	2.421	2.406	30
Unknown	0.446	0.470	0.458	4.3

TYPICAL CALIBRATOR CURVE



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the standard curve by determining the resulting concentration of the mean OD of Calibrator A (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the Direct TSH ELISA kit is **0.1 µIU/mL.**

SPECIFICITY (CROSS-REACTIVITY)

The specificity of the Direct TSH ELISA kit was determined by measuring the apparent TSH values of the following compounds:

Substance	Concentration Range	Apparent TSH Value (µIU/mL)
hCG Calibrated against WHO 1st IS 75/537	10,000-50,000 IU/L	< 0.15
hFSH Calibrated against WHO 1st IS 83/575	1000-4000 IU/L	< 0.15
hLH Calibrated against WHO 2nd IS 80/575	100-500 IU/L	< 0.15

INTRA-ASSAY PRECISION

Three samples were assayed ten times each on the same calibrator curve. The results (in µIU/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.52	0.07	13.3
2	1.54	0.10	6.4
3	9.27	0.72	7.7

INTER-ASSAY PRECISION

Three samples were assayed ten times over a period of four weeks. The results (in µIU/mL) are tabulated below:

Sample	Mean	SD	CV %
1	0.78	0.07	8.3
2	8.03	0.99	12.3
3	25.42	3.26	12.8

RECOVERY

Spiked samples were prepared by adding defined amounts of TSH to three patient serum samples. The results (in µIU/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1 Unspiked	1.92	-	-
+ 0.25	2.31	2.17	106.5
+ 3.0	5.12	4.92	104.1
+ 7.5	10.26	9.42	108.9
2 Unspiked	2.01	-	-
+ 0.25	2.27	2.26	100.4
+ 3.0	5.10	5.01	101.8
+ 7.5	9.36	9.51	98.4
3 Unspiked	2.02	-	-
+ 0.25	2.35	2.27	103.5
+ 3.0	4.87	5.02	97.0
+ 7.5	8.57	9.52	90.0

LINEARITY

Three patient serum samples were diluted with calibrator A. The results (in µIU/mL) are tabulated below:

Sample	Obs. Result	Exp. Result	Recovery %
1	9.36	-	-
1:2	4.53	4.68	96.8
1:4	2.31	2.34	98.7
1:8	1.08	1.17	92.3
2	10.89	-	-
1:2	5.65	5.45	103.7
1:4	2.96	2.72	108.8
1:8	1.32	1.36	97.1
3	11.85	-	-
1:2	6.03	5.93	101.7
1:4	2.43	2.96	82.1
1:8	1.18	1.48	79.7

COMPARATIVE STUDY

The Direct TSH ELISA kit (Kit A) was compared with two other competitors ELISA kits (Kit B and Kit C). The results (in µIU/mL) are tabulated below:

Group	N	Kit A Mean	Kit B Mean	Kit C Mean
Random Males and Females	27	2.97	3.36	2.89

EXPECTED NORMAL VALUES

As for all clinical assays each laboratory should collect data and establish their own range of expected normal values.

Group	Range (µIU/mL)
Normal	0.3-5
Hyperthyroid	< 0.15
Hypothyroid	> 5.7

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