Human TSH ELISA Kit

to quantitatively determine Human TSH in Human Blood Serum or Plasma

INSTRUCTION MANUAL

FOR ELISA KIT No: LT5096TEKKBA

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INTENDED USE

This kit is used to quantitatively determine the Human TSH in the sample of Human Blood Serum or Plasma. For *in vitro* use only.

MANUAL VERSION 1.02X

BACKGROUND

Thyroid-stimulating hormone (also known as thyrotropin, thyrotropic hormone, TSH, or hTSH for human TSH) is a pituitary hormone that stimulates the thyroid gland to produce thyroxine (T4), and then triiodothyronine (T3) which stimulates the metabolism of almost every tissue in the body. It is a glycoprotein hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland, which regulates the endocrine function of the thyroid. In 1916, Bennett M. Allen and Philip E. Smith found that the pituitary contained a thyrotropic substance.

PRINCIPLE

This test is based on two-site sandwich enzyme immunoassay principle. Tested specimen is placed into the microwells coated by specific murine monoclonal to β chain human TSH-antibodies. Antigen from the specimen is captured by the antibodies coated onto the microwell surface. Second antibodies – murine monocnoclonal to (Fab2)-fragment of β chain human TSH, labelled with peroxidase enzyme, are then added into the microwells. After washing procedure, the remaining enzymatic activity bound to the microwell surface is detected and quantified by addition of chromogen-substrate mixture, stop solution and photometry at 450 nm. Optical density in the microwell is directly related to the quantity of the measured analyte in the specimen

ASSAY RESTRICTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.

- If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

MATERIALS SUPPLIED IN THIS KIT

Description		Qty	Units	Colour code	Stability of opened/diluted components
TSH EIA strips, 8x12 wells	polystyrene microwells coated with murine monoclonal to β chain human TSH	1	pcs		until exp.date
Calibrator set, 0.8 ml each, zero calibrator C1 – 2 ml The set contains 6 calibrators: 0; 0.2; 1; 5; 10, 20 mIU/l	human TSH diluted in phosphate buffered of preselected horses serum, casein solution, preservative – 0,1% phenol; also contains bright red dye	6	pcs	red (C1 – colourless)	2 months
Control serum (0.8 ml)	dilution of preselected human serum, with high content of TSH with casein solution; preservative – 0,1% phenol, colourless	1	pcs	colourless	2 months
Conjugate, 11 ml	aqueous solution of murine monocnoclonal to (Fab2)-fragment of β chain human TSH coupled with horseradish peroxidase diluted on phos-phate buffered solution with casein from bovine milk and detergent (Tween-20), contains 0,1% phenol as preservative and bright blue dye	1	pcs	blue	until exp.date
Substrate solution, 11 ml	ready-to-use single-component tetramethylbenzidine (TMB) solution.	1	pcs	colourless	until exp.date
Washing solution concentrate 21x, 22 ml	aqueous solution of sodium chloride and detergent (Tween 20), contains proClin300 as a preservative	1	pcs	colourless	Concentrate – until exp.date Diluted washing solution – 1 month at 2...+8°C or 5 days at RT
Stop solution, 11 ml	5,0% vol/vol solution of sulphuric acid	1	pcs	colourless	until exp.date
Plate sealing tape		2	pcs		N/A
Instruction TSH EIA, English		1	pcs		N/A
QC data sheet TSH EIA		1	pcs		N/A

MATERIALS REQUIRED BUT NOT SUPPLIED

1. 37 °C incubator 2. Microplate reader 3. Precision pipettes and tips
4. Distilled water 5. Disposable tubes for sample dilution 6. Absorbent paper

STORAGE CONDITIONS

The unopened kit should be stored at 2-8˚C for 1 year. Immediately after use remaining reagents should be returned to cold storage at 4˚C

PRECAUTIONS

Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer. 2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at 2-8 °C in their pouch with the desiccant provided. 3. Mix all reagents before using. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25 °C).

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

• Substrate solution should remain colourless until added to the plate. Keep Substrate solution protected from light. Substrate solution should change from colorless to gradations of blue.

• Stop Solution should be added to the plate in the same order as the Substrate solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate solution.

SAMPLE COLLECTION & STORAGE

This kit is intended for use with serum or plasma (ACD- or heparinized). Grossly hemolytic, lipemic, or turbid samples should be avoided. Specimens may be stored for up to 48 hours at +2…+8 °C before testing. For a longer storage, the specimens should be frozen at -20 °C or lower. Repeated freezing/ thawing should be avoided. The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum - Use a serum separator tube and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20˚C. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20˚C. Avoid repeated freeze-thaw cycles. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Samples should be aliquoted and must be stored at -20 ˚C. If samples are to be used within 24 hours, they may be stored at 2 to 8 ˚C. Avoid repeated freeze-thaw cycles. Prior to assay,

the frozen sample should be brought to room temperature slowly and mixed gently.

REAGENT PREPARATION

—All reagents (including unsealed microstrips) should be allowed to reach room temperature (+18…+25 °C) before use.

—All reagents should be mixed by gentle inversion or vortexing prior to use. Avoid foam formation.

—It is recommended to spin down shortly the tubes with calibrators on low speed centrifuge.

—Prepare washing solution from the concentrate BUF WASH 21X by 21 dilutions in distilled water.

—--It is recommended that pipetting of all calibrators and samples should be completed within 3 minutes.

ASSAY PROCEDURE

1 Put the desired number of microstrips into the frame; allocate 14 wells for the calibrators CAL 1–6 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.

2 If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using (zero calibrator). Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

3 Dispense 100 µl of CONJ HRP into the wells.

4 Pipet 50 µl of calibrators CAL 1–6, control samples CONTROL and unknown samples into the wells. Cover the wells by plate adhesive tape (included into the kit).

5 Incubate 60 minutes at 37 °C.

6 Prepare washing solution by 21x dilution of washing solution concentrate (BUF WASH 21X) by distilled water. Wash the strips 5 times.

7 Dispense 100 µl of SUBS TMB into the wells

8 Incubate 10-20 minutes at +18…+25 °C

9 Dispense 100 µl of STOP into the wells.

10 Measure OD (optical density) at 450 nm.

11 Set photometer blank on first calibrator

12 Apply point-by-point method for data reduction.

FOR Pregnant Women:

1 Put the desired number of microstrips into the frame; allocate 14 wells for the calibrators CAL 1–6 and control samples CONTROL and two wells for each unknown sample. DO NOT REMOVE ADHESIVE SEALING TAPE FROM UNUSED STRIPS.

2 If suggested analyte concentration in the sample exceeds the highest calibrator, additionally dilute this sample accordingly, using zero calibrator. Use of other buffers or reagents for sample dilution may lead to incorrect measurement.

3 Pipet 50 µl of calibrators CAL 1–6, control samples CONTROL and unknown samples into the wells. Cover the wells by plate adhesive tape (included into the kit).

4 Incubate 30 minutes at 37 °C.

5 Prepare washing solution by 21x dilutions of washing solution concentrate BUF WASH 21X by distilled water. Minimal quantity of washing solution should be 250 µl per well. Wash strips 3 times

6 Dispense 100 µl of CONJ HRP into the wells. Cover the wells by plate adhesive tape.

7 Incubate 30 minutes at 37 °C.

8 Wash the strips 5 times.

9 Dispense 100 µl of SUBS TMB into the wells

10 Incubate 10–20 minutes at +18…+25 °C

11 Dispense 100 µl of STOP into the wells.

12 Measure OD (optical density) at 450 nm.

13 Set photometer blank on first calibrator

14 Apply point-by-point method for data reduction.

EXPECTED VALUES AND CHARACTERISTICS

The following normal range is recommended (see below). NOTE: the patients that have received murine monoclonal antibodies for radioimaging or immunotherapy develop high titered anti-mouse antibodies (HAMA). The presence of these antibodies may cause false results in the present assay. Sera from HAMA positive patients should be treated with depleting adsorbents before assaying.

Healthy donors: 0.3 to 4.0

PERFORMANCE CHARACTERISTICS

Analytical specificity / Cross reactivity

HCG <0.1 [Analyte Cross-reactivity, % wt/wt]

LH <0.1 [Analyte Cross-reactivity, % wt/wt]

FSH <0.1 [Analyte Cross-reactivity, % wt/wt]

Sensitivity of the assay was assessed as being 0.08 mIU/l.

Linearity was checked by assaying dilution series of 5 samples with different TSH concentrations. Linearity percentages obtained ranged within 90 to 110%.

Recovery was estimated by assaying 5 mixed samples with known TSH concentrations. The recovery percentages ranged from 90 to 110%

CALCULATION

Calculate the mean absorbance values (OD450) for each pair of calibrators and samples. Plot a calibration curve on graph paper: OD versus total TSH concentration. Determine the corresponding concentration of total TSH in unknown samples from the calibration curve. Manual or computerized data reduction is applicable on this stage. Point-by-point or linear data reduction is recommended due to non-linear shape of curve.

VALIDITY & STORAGE: 12 months (at 2-8℃, unopened).