TSH Elisa

KAPDB4080

DIAsource ImmunoAssays S.A. - Rue du Bosquet, 2 - B-1348 Louvain-la-Neuve - Belgium

LOT : 110715/1

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For the direct quantitative determination of Thyroid Stimulating Hormone by enzyme immunoassay in human serum.

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IN VITRO DIAGNOSTIC

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INTENDED USE

For the direct quantitative determination of Thyroid Stimulating Hormone by enzyme immunoassay in human serum.
For *in vitro* diagnostic use only.

PRINCIPLE OF THE TEST

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 microwell plate and another monoclonal antibody specific for a different region of TSH is conjugated to horse radish peroxidase (HRP). TSH from the sample and calibrators 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 calibrators is used to plot a calibration curve from which the amount of TSH in patient samples and controls can be directly read.

CLINICAL APPLICATIONS

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. However, the β subunit 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.

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 control 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 control do not reflect established ranges.
9. When reading the microplate, the presence of bubbles in the microwells 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, calibrator 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 0 may be used to dilute any high serum samples. The use of any other reagent may lead to false results.
5. The results obtained with this kit should never be used as the sole basis for clinical diagnosis. For example, the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products has the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should include all aspects of a patient's background including the frequency of exposure to animals/products if false results are suspected.
6. Some individuals may have antibodies to mouse protein that can possibly interfere in this assay. Therefore, the results from any patients who have received preparation of mouse antibodies for diagnosis or therapy should be interpreted with caution.

SAFETY CAUTIONS AND WARNINGS

POTENTIAL BIOHAZARDOUS MATERIAL

Human serum that may be used in the preparation of the calibrators and control has been tested and found to be non-reactive 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. However no test method 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. Microwell plate reader with a filter set at 450nm and an upper OD limit of 3.0 or greater* (see assay procedure step 10).

REAGENTS PROVIDED

Mouse Anti-TSH Antibody Coated Microwell Plate-Break Apart Wells - Ready To Use.
Contents: One 96 well (12x8) monoclonal antibody-coated microwell plate in a resealable pouch with desiccant.
Storage: Refrigerate at 2-8°C
Stability: 12 months or as indicated on label.

Ab | HRP | CC — **Mouse Anti-TSH Antibody-Horseradish Peroxidase (HRP) Conjugate Concentrate** – 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.

CAL | N — **TSH Calibrators** - Ready To Use. N = 0 to 5

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.
1µIU of the calibrator is equivalent to 1 µIU of the 2nd IIS 80/558.
*Listed below are approximate concentrations, please refer to vial labels for exact concentrations.

Calibrator	Concentration	Volume/Vial
Calibrator 0	0 µIU/mL	2.0 mL
Calibrator 1	0.2 µIU/mL	0.5 mL
Calibrator 2	1 µIU/mL	0.5 mL
Calibrator 3	5 µIU/mL	0.5 mL
Calibrator 4	15 µIU/mL	0.5 mL
Calibrator 5	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 calibrators should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

CONTROL — **Control** - Ready To Use.

Contents: One vial containing TSH in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of TSH. Refer to vial label for expected value and 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 control should be used within 14 days or aliquoted and stored frozen. Avoid multiple freezing and thawing cycles.

WASH | SOLN | CONC — **Wash Buffer Concentrate** – 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.

ASS | BUF — **Assay Buffer** - Ready To Use.

Contents: One vial 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.

CHROM | TMB — **TMB Substrate** - Ready To Use.

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

STOP | SOLN — **Stopping Solution** - Ready To Use.

Contents: One vial 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 solution of the anti-TSH-HRP conjugate and wash buffer.
2. Remove the required number of microwell 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 microwell plate 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 415 nm filter may be substituted. The optical densities will be lower, however, this will not affect the results of patient/control samples.

CALCULATIONS

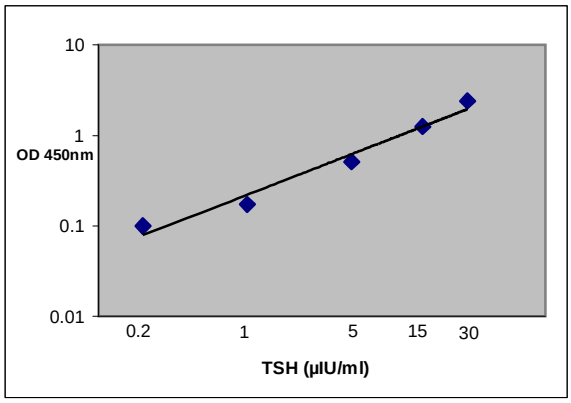
1. Calculate the mean optical density of each calibrator duplicate.
2. Calculate the mean optical density of each unknown duplicate.
3. Subtract the mean absorbance value of the "0" calibrator from the mean absorbance values of the calibrators, control and serum samples.
4. Draw a calibrator curve on 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 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 0 at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

TYPICAL TABULATED DATA

Calibrator	OD 1	OD 2	Mean OD	Value (µIU/ml)
0	0.071	0.073	0.072	0
1	0.100	0.099	0.100	0.2
2	0.177	0.171	0.174	1
3	0.492	0.527	0.510	5
4	1.270	1.254	1.262	15
5	2.391	2.421	2.406	30
Unknown	0.446	0.470	0.458	4.3

TYPICAL CALIBRATION CURVE

Sample curve only. **Do not** use to calculate results.



PERFORMANCE CHARACTERISTICS

SENSITIVITY

The lower detection limit is calculated from the calibration curve by determining the resulting concentration of the mean OD of Calibrator 0 (based on 10 replicate analyses) plus 2 SD. Therefore, the sensitivity of the DIAsource 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 83/575	1000-4000 IU/L	<0.15
hLH Calibrated against WHO 2nd IS 80/552	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 0. 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 STUDIES

The DIAsource Direct TSH ELISA kit (Kit A) was compared with two other competitor ELISA kits (Kit B and Kit C)
(Kit B). 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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	Used symbols
	Consult instructions for use
	Storage temperature
	Use by
LOT	Batch code
REF	Catalogue number
CONTROL	Control
I V D	In vitro diagnostic medical device
	Manufacturer
	Contains sufficient for <n> tests
WASH SOLN CONC	Wash solution concentrated
CAL 0	Zero calibrator
CAL N	Calibrator #
CONTROL N	Control #
Ag 125I	Tracer
Ab 125I	Tracer
Ag 125I CONC	Tracer concentrated
Ab 125I CONC	Tracer concentrated
	Tubes
INC BUF	Incubation buffer
ACETONITRILE	Acetonitrile
SERUM	Serum
DIL SPE	Specimen diluent
DIL BUF	Dilution buffer
ANTISERUM	Antiserum
IMMUNOADSORBENT	Immunoadsorbent
DIL CAL	Calibrator diluent
REC SOLN	Reconstitution solution
PEG	Polyethylene glycol
EXTR SOLN	Extraction solution
ELU SOLN	Elution solution
GEL	Bond Elut Silica cartridges
PRE SOLN	Pre-treatment solution
NEUTR SOLN	Neutralization solution
TRACEUR BUF	Tracer buffer
	Microtiterplate
Ab HRP	HRP Conjugate
Ag HRP	HRP Conjugate
Ab HRP CONC	HRP Conjugate concentrate
Ag HRP CONC	HRP Conjugate concentrate
CONJ BUF	Conjugate buffer
CHROM TMB CONC	Chromogenic TMB concentrate
CHROM TMB	Chromogenic TMB solution
SUB BUF	Substrate buffer
STOP SOLN	Stop solution
INC SER	Incubation serum
BUF	Buffer
Ab AP	AP Conjugate
SUB PNPP	Substrate PNPP
BIOT CONJ CONC	Biotin conjugate concentrate
AVID HRP CONC	Avidine HRP concentrate
ASS BUF	Assay buffer
Ab BIOT	Biotin conjugate
Ab	Specific Antibody
SAV HRP CONC	Streptavidin HRP concentrate
NSB	Non-specific binding
2nd Ab	2nd Antibody
ACID BUF	Acidification Buffer
DIST	Distributor