INSTRUCTION MANUAL

IVD

(April 01, 2014)

Medizym® TSH hs

- 96 determinations -

REF **3808**

CE

Third generation enzyme immunoassay for the high sensitive determination of thyrotropin (TSH) in human serum

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IFU symbols non-radioactive assays MEDIPAN GMBH

IVD	In vitro diagnostic device	CE	EC Declaration of Conformity
REF	Catalogue number	LOT	Batch code
	Expiry date		Manufactured by
	Consult accompanying documents		Consult operating instruction
	Store at		Biological risk
MP	Coated microtiterplate (96 wells)	CONJ	Conjugate
WASHB	Wash buffer	CONTROL	Control serum
SUB	Substrate	CAL	Calibrators
STOP	Stop solution		

INTENDED USE

The thyroid stimulating hormone (thyrotropin or TSH) is a glycoprotein with a molecular weight of 28 kDa, secreted by the adenohypophysis.

The synthesis and the release of TSH are controlled by the circulatory level of thyroid hormones triiodothyronine (T_3) and thyroxin (T_4) and by the hypothalamic thyrotropin releasing hormone (TRH). Thyroid hormones regulate the secretion of TSH by a negative feed-back mechanism. An elevation of T_3 or T_4 will suppress, and their decrease will, in turn, increase the level of TSH in serum. The increased concentration of TSH in the serum is the earliest and best indicator of primary hypothyroidism.

The determination of TSH by immunoassay methods plays a crucial role in the diagnosis of thyroid disorders and in the evaluation of the functional integrity of the hypothalamic-pituitary axis.

The outstanding sensitivity of the present 3rd generation assay Medizym® TSH hs makes it particularly suitable for the measurement of subnormal human TSH levels, a key to both the diagnosis and treatment follow-up of hyperthyroid patients.

LITERATURE

1. Laboratory Medicine Practice Guidelines: Laboratory Support for the Diagnosis and Monitoring of Thyroid Disease Section 3: Thyroid Tests for the Clinical Biochemist and Physician; C. Thyrotropin/Thyroid Stimulating Hormone (TSH) Measurement, Guidelines Committee, Thyroid 2003;13:33-44

2. Spencer CA, Takeuchi M, Kazarosyan M, MacKenzie F, Beckett GJ, and Wilkinson E. Interlaboratory / intermethod differences in functional sensitivity of immunoradiometric assays of thyrotropin (TSH) and impact on reliability of measurement of subnormal concentrations of TSH. Clin Chem 1995; 41: 367-74.

3. Spencer CA, Takeuchi M, and Kazarosyan M. Current status and performance goals for serum thyrotropin (TSH) assays. Clin Chem 1996; 42(1): 140-5.

PRINCIPLE of the TEST

Medizym® TSH hs is a one-step enzyme immunoassay (sandwich). The HRP- conjugated antibody (signal) and the capture antibody coated on the plate react simultaneously with the TSH (antigen) present in standards or samples.

After an incubation of 1 hour, a specific sandwich complex (capture antibody-TSH-conjugated antibody) is resulting - immobilised on the surface of the wells. The unbound fraction will be removed by washing steps.

The substrate solution is added into each well for colour development. The intensity of the colour in the well is proportional to the concentration of TSH in serum.

Optical density (OD) should be measured at 450 nm and 405 nm. With the obtained values two standard curves are elaborated (concentration versus OD): A). concentration of calibrators 0-5 (0-2,5 µIU/ml) versus OD at 450 nm; B). Concentration of calibrators 4-7 (0,75-15 µIU/ml) versus OD at 405 nm. TSH concentrations of the unknown samples must be read-off as follow: samples with an expected TSH concentration between 0 and 2.5 µIU/ml on curve at 450 nm; samples with an expected TSH concentration between 2.5 and 15.0 µIU/ml on curve at 405 nm.

If expected TSH concentration in the sample is higher than calibrator 7 (15 µIU/ml) samples should be diluted 10-fold with calibrator 0 (0 µIU/ml).

PATIENT SAMPLES

Specimen collection and storage

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Do not use plasma, lipaemic or haemolytic samples, or serum containing sodium azide.
The samples may be kept at 2 - 8 °C for up to two days. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep them at - 20 °C.

TEST COMPONENTS for 96 DETERMINATIONS

A MP	**Microtiter plate**, 12 breakable strips per 8 wells (total 96 individual wells) coated with anti-hTSH		1 Reusable plastic bag
B WASHB	**Wash buffer** to dilute with 450 ml distilled water before use		1 vial 50 ml each concentrated
D CONJ	**Conjugate** containing signal antibody coupled with horse radish peroxidase (HRP)		1 vial 14 ml ready for use
E SUB	**Substrate** 3,3',5,5'-tetramethylbenzidine (TMB) in citrate buffer containing hydrogen peroxide		1 vial 14 ml ready for use
F STOP	**Stop solution** 1.0 N HCl		1 vial 14 ml ready for use
0 CAL	**Calibrator** (TSH-free serum) 0.0 µIU/ml	L	4 vials 2.5 ml each ready for use
1 - 7 CAL	**Calibrators** (diluted serum) 2 vials for each concentration conc.: see leaflet enclosed	L	7x 2 vials 0.5 ml each ready for use
CI, CII CONTROL	**Control sera** (diluted serum) conc.: see leaflet enclosed	L	2x 2 vials 0.5 ml each ready for use

Materials required

- Precision pipettes 10 - 100 µl
- Multi-channel pipette 300 µl
- Disposable pipette tips
- 8-channel wash comb with a vacuum pump or microtiter plate washer
- Microtiter plate reader with optical filters for 450 nm, 405 nm (or 492 nm alternatively) and 620 nm (or 690 nm alternatively)
- Graduated cylinders
- Distilled water
- Absorbent paper or paper towel
- microplate shaker-thermostat (able to maintain a temperature of +37°C and a shaking speed of up to 700 rpm) can be purchased from MEDIPAN

Size and storage

Medizym® TSH hs has been designed for 96 determinations. This is sufficient for the analysis of 38 unknown samples as well as for calibrators and the control serum, all assayed in duplicates.

The expiry date of each component is reported on its respective label; that of the complete kit is printed on the box labels.

Upon receipt, all components of the Medizym® TSH hs have to be kept at 2 - 8 °C, preferably in the original kit box.

Preparation before use

Allow all components to reach room temperature prior to use in the assay for at least 30 minutes. Take care to agitate serum samples gently in order to ensure homogeneity.

Note: ***Samples from patients who were stimulated with recombinant human TSH or underwent withdrawal of L-thyroxine in case of differentiated thyroid cancer (DTC) before radioiodine treatment have to be diluted (e.g. 1 : 10) using Calibrator (0) prior to assay.***

The same should be done with samples from patients who were stimulated with thyrotropin releasing hormone (TRH testing) in suspicious cases of subclinical hypothyroidism.

A) The microtiter plate is in a reclosable foil bag. The plate consists of a frame and strips with breakable wells. Allow the sealed microplate to reach room temperature before opening. Unused wells should be stored refrigerated and protected from moisture in the original bag carefully resealed.

B) Prepare a sufficient amount of washing solution by diluting the concentrated wash buffer 10 times (1 + 9) with distilled water. For example, dilute 10 ml of the concentrate with 90 ml of distilled water. The washing solution prepared is stable up to 30 days at 2 - 8 °C or.

CI, CII) D) E) store at 2 - 8 °C for not more than 4 weeks after opening.

0, 1-7) store at 2 - 8 °C for not more than 4 weeks after opening.

Crystallisation of undiluted wash buffer may occur. Crystals can be dissolved by warming up at 37 °C.

Avoid exposure of the TMB substrate solution to light!

ASSAY PROCEDURE

- **Duplicates are recommended.**

1. Bring all reagents to room temperature before use. Mix gently, avoid foam.
2. Dispense
   50 µl calibrators (0 - 7)
   50 µl control sera (CI, CII) and patient samples
   into the respective wells.
3. Add **100 µl** of conjugate (D) to each well.
4. Cover the plate and incubate for **1 hour at 37 °C** while shaking at **700 rpm**.
5. Aspirate and wash the wells **5 times** with **300 µl** of washing solution (made from B). Strike the wells sharply onto absorbent paper towels to remove all residual water droplets.
6. Add **100 µl** of substrate (E) to each well.
7. Cover the plate and incubate **15 min in the dark at 37 °C** while shaking at **700 rpm**.
8. Add **100 µl** of stop solution (F) to each well and shake 5 sec. (be sure that all the blue colour changes to yellow colour completely).
9. Read the optical density at **450 nm** (0 - 7.5 µIU/ml) and **405 nm** or 492 nm (up to 15.0 µIU/ml) versus 620 nm or 690 nm within **20 min** after adding the stop solution.

Please note that the washing procedure is crucial. Insufficient washing is linked to poor precision and will result in falsely elevated OD readings.

DATA PROCESSING

The standard curve is established by plotting the mean OD-values of the calibrators 0 - 7 on the ordinate, y-axis versus their respective TSH concentrations on the abscissa, x-axis.

TSH concentrations of the unknown samples are directly read off in µIU/ml against the respective OD values.

Medizym® TSH hs may be used also with Computer Assisted Analysis using software able to calculate curves with spline smoothing fit.

TYPICAL EXAMPLE

Do not use for evaluation!

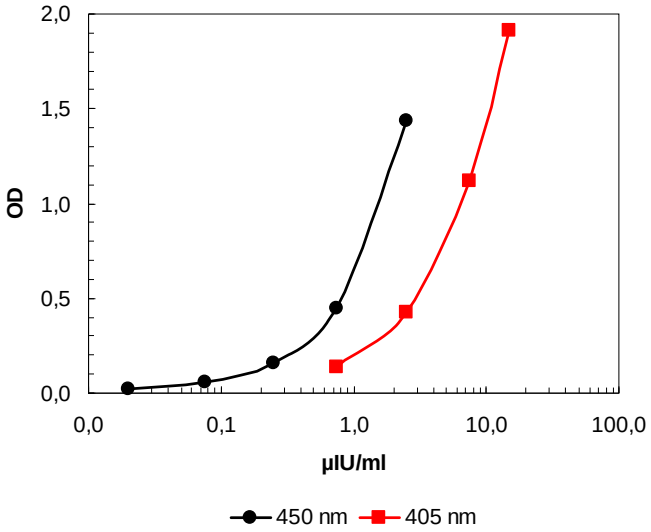
Sample	OD (a) 450 nm	OD (b) 450 nm	OD (mean)	µIU/ml
Calibrator **0**	0.014	0.013	0.014	**0.000**
Calibrator **1**	0.025	0.025	0.025	**0.020**
Calibrator **2**	0,059	0,056	0.058	**0.075**
Calibrator **3**	0.160	0.157	0.158	**0.250**
Calibrator **4**	0.454	0.439	0.446	**0.750**
Calibrator **5**	1.439	1.428	1.434	**2.500**
Control **CI**	0.726	0.728	0.727	**1.345**

Sample	OD (a) 405 nm	OD (b) 405 nm	OD (mean)	µIU/ml
Calibrator **4**	0.141	0.139	0.140	**0.750**
Calibrator **5**	0.428	0.429	0.428	**2.500**
Calibrator **6**	1.128	1.116	1.122	**7.500**
Calibrator **7**	1.934	1.896	1.915	**15.000**
Control **CI**	0.219	0.222	0.220	**1.285**

The above mentioned standard concentrations are only an example for a typical standard curve. They can change from lot to lot.

STANDARD CURVE

Typical example



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Standardization

The included TSH standard curve is calibrated against the actual International Reference Preparation: 2nd IRP 80/558

Criteria of validation

Specimens with an expected TSH concentration between 0 and 2.5 µIU/ml should be read-off on curve at 450 nm; samples with an expected TSH concentration between 2.5 and 15.0 µIU/ml on curve at 405 nm.

REFERENCE VALUES

According to the Guidelines Development of the National Academy of Clinical Biochemistry (NACB) all laboratories should establish their own reference interval independent of the manufacturer's recommendations. According to this, the following reference values should be for orientation only.

In normal, healthy persons, the serum TSH values range from 0.3 to 3.5 µIU/ml.

In clinically euthyroid patients, serum TSH is normally below 3.5 µIU/ml. This applies also to patients on thyroid hormone replacement or on and after antithyroid drug treatment, respectively. Furthermore, patients suffering from severe non-thyroidal illness (NTI) etc., show frequently subnormal TSH values.

Sub-clinical hypothyroidism is associated with basal TSH values above 3 µIU/ml, whereas serum TSH above 5 µIU/ml is more and more indicative for overt hypothyroidism.

Subnormal TSH values (< 0.3 µIU/ml) are observed in sub-clinical hyperthyroidism. In thyrotoxicosis, however, the TSH is suppressed (< 0.1 µIU/ml).

CHARACTERISTIC ASSAY DATA

Sensitivity

The analytical sensitivity is 0.007 µIU/ml calculated as mean (n = 12) of a TSH free serum + 3 SD.

The functional sensitivity is determined to be 0.015 µIU/ml (20 % Inter-assay CV):

Intra-assay (n= 8)			Inter-assay (n= 5)		
Sample no.	mean (µIU/ml)	CV (%)	Sample no.	mean (µIU/ml)	CV (%)
1	0.05	4	5	0.02	7
2	0.5	5	6	0.5	3
3	2.4	3	7	3.4	5
4	5.6	1	8	12.8	2

Specificity

The following hormones were tested: hCG, LH, FSH and no cross reactivity was detected. For Medizym® TSH hs kit **high dose hook effect** was not detected for concentrations up to 800 µIU/ml.

LIMITATIONS of the METHOD

Any clinical diagnosis should not be based on the result of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Medizym® TSH hs

INCUBATION SCHEME

Step	Activity	Material	CAL	Control sera	Patients 1, 2 etc.
1	Bring to room temperature	All test components	**CAL 0 - 7**	**CI, CII**	**1, 2, …**
2	Pipette	Calibrators (0-7) Control (C) Samples	50 µl	50 µl	50 µl
3	Pipette	Conjugate (D)	100 µl	100 µl	100 µl
4	Cover and incubate		**1 hour at 37 °C with shaking (700 rpm)**		
5	Aspirate or decant Pipette Aspirate or decant	Washing solution (made from B)	put sharply onto absorbent tissue 5 x 300 µl put sharply onto absorbent tissue	5 x 300 µl	5 x 300 µl
6	Pipette	Substrate (E)	100 µl	100 µl	100 µl
7	Cover and incubate		**15 min at 37 °C while shaking in the dark (700 rpm)**		
8	Pipette and shake	Stop solution (F)	100 µl	100 µl	100 µl
9	Measure OD		at 450 nm and 405 nm (or 492 nm) versus 620 nm (or 690 nm) within 20 min		

SAFETY PRECAUTIONS

- **This kit is for in vitro use only**. Follow the working instructions carefully. This instruction manual is valid only for the present kit with the given composition. An exchange of single components is not in agreement with CE regulations.
- The expiration dates stated on the respective labels are to be observed. The same relates to the stability stated for re-constituted reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept at 2 - 8 °C before use in the original shipping container.
- Some of the reagents contain small amounts of Kathon MW as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.

- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.

- Since the kit contains potentially hazardous materials, the following precautions should be observed:
  - Do not smoke, eat or drink while handling kit material,
  - Always use protective gloves,
  - Never pipette material by mouth,
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.

In any case GLP should be applied with all general and individual regulations to the use of this kit.