Instructions for Use

TSH 1h (CT) IRMA

IVD

CE

REF **RIA-4200**

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(160711, v2)

Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.
Utilisez seulement la version valide des Instructions d'utilisation fournies avec le kit.

Table of Contents

1 INTRODUCTION

Thyroid-stimulating hormone (TSH) or human thyrotropin is a glycoprotein hormone of approximate molecular weight of 28,000 Da, secreted by thyreotrope cells of the anterior pituitary gland. hTSH is composed of two non covalently bound distinct subunits designated α and β.The α-subunit is common to the other glycoprotein hormones: follicle stimulating hormone (hFSH), luteinizing hormone (hLH) and

chorionic gonadotropin (hCG). The beta-subunit determines both the biological and immunological specificity and allows the recognition and the differentiation of hTSH from other glycoprotein hormones by the antibody.

The main function of hTSH is the regulation of synthesis and release of the thyroid hormones. The secretion and the liberation of hTSH are stimulated by the hypothalamic tripeptide TRH (TSH-Releasing Hormone) and are controlled by serum concentrations of the thyroid hormones: thyroxine (T4) and triiodthyronine (T3) through a negative feedback mechanism: in the thyreotrope cells, T4 is desiodinated in T3 which directly inhibits hTSH secretion.

When the hypothalamo-hypophyso-thyroid axis is normal, thyroid hormone insufficiency is associated with a response of the thyreoprope cells to TRH, and an increased secretion of hTSH. In contrast, when thyroid hormones are available in excess, response to TRH is reduced and hTSH secretion is suppressed. Hypothyroidism and hyperthyroidism are associated with pathologies if diverse origins of the hypothalamo-hypophyso-thyroid axis, reflected by the concentrations in hTSH, thyroid hormones, and the hTSH response to the exogenous TRH test: see the following table.

Until now, hTSH measurement was the most sensitive indicator for the diagnosis of primary hypothyroidism with an increased secretion of hTSH and a decrease of thyroid hormone concentrations.

Today, the use of monoclonal antibodies increases the sensitivity of the test by immunoradiometric sandwich assay and allows a better discrimination between hyperthyroid and euthyroid population with a decrease in the level.

2 PRINCIPLE OF THE ASSAY

The immunoradiometric assay of thyroid-stimulating hormone (TSH) is a “sandwich” type assay. Mouse monoclonal antibodies directed against two different epitopes of TSH and hence not competing are used. The samples or standards are incubated in tubes coated with the first monoclonal antibody in the presence of the second monoclonal antibody labeled with iodine 125. After incubation, the content of tubes is aspirated and the tubes are rinsed so as to remove unbound I-125-labeled antibody. The bound radioactivity is then determined in a gamma counter. The TSH concentrations in the samples are obtained by interpolation from the standard curve. The concentration of TSH in the samples is directly proportional to the radioactivity.

3 WARNING AND PRECAUTIONS

3.1 General remarks

- The vials with calibrators and controls should be opened as shortly as possible to avoid excessive evaporation.
- Do not mix the reagents from kits of different lots.
- A standard curve must be included with each assay.
- It is recommended to perform the assay in duplicate.
- Each tube must be used only once.

3.2 Basic rules of radiation safety

The purchase, possession, utilization, and transfer of radioactive material is subject to the regulations of the country of use. Adherence to the basic rules of radiation safety should provide adequate protection:

- No eating, drinking, smoking or application of cosmetics should be carried out in the presence of radioactive materials.
- No pipeting of radioactive solutions by mouth.
- Avoid all contact with radioactive materials by using gloves and laboratory overalls.
- All manipulation of radioactive substances should be done in an appropriate place, distant from corridors and other busy places.
- Radioactive materials should be stored in the container provided in a designated area.
- A record of receipt and storage of all radioactive products should be kept up to date.
- Laboratory equipment and glassware which are subject to contamination should be segregated to prevent cross-contamination of different radioisotopes.
- Each case of radioactive contamination or loss of radioactive material should be resolved according to established procedures.
- Radioactive waste should be handled according to the rules established in the country of use.

3.3 Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide may react with lead, copper or brass to form explosive metal azides. Dispose of the reagents by flushing with large amounts of water through the plumbing system.

3.4 Materials of human origin

The materials of human origin, contained in this kit, were found negative for the presence of antibodies to HIV 1 and HIV 2, antibodies to HCV, as well as of Hepatitis B surface antigen (HBsAg). However, they should be handled as if capable of transmitting disease. No known test method can offer total assurance that no virus is present. Handle this kit with all necessary precautions.

All serum and plasma samples should be handled as if capable of transmitting hepatitis or AIDS and waste should be discarded according to the country rules.

Safety Data Sheet is available at drg@drg-diagnostics.de.

4 REAGENTS PROVIDED

The TSH Assay contains sufficient reagents for 100 determinations.

All reagents of the kit are stable until the expiry date indicated on the kit label, if stored at 2 °C - 8 °C.

After reconstitution / use, the reagents are stable until the expiry date of the kit.

Do not mix reagents from different lots.

Allow reagents to come to room temperature.

SORB | CT Anti-TSH antibody-coated tubes, ready-to-use:

2 x 50 tubes

Anti-TSH | I-125 I-125- anti-TSH Ab-Tracer, ready-to-use:

1 vials each contains 11.0 mL of I-125-labeled immunglobulins in buffer containing bovine serum albumin. Activity per vial: < 515 kBq, at the date of manufacture,

Preservative: sodium azide <0.1% (see § Precautions), and a dye.

CAL TSH-Standards: 0 – 6, ready-to-use:

1 Set with 7 vials each contains 1.0 mL bovin serum (see § precautions)

Concentration from 0 to 50 mIU/L.

The exact concentration is indicated on the QC datasheet.

The standards were calibrated against the international standard, WHO 3rd IS 2003 81/565.

Preservative: sodium azide <0.1% (see § precautions).

CONTROL Controls 1 &2, lyophilised:

2 vials, each contains TSH lyophilised in bovine serum (see § Precautions).

Reconstitute each vial with 1.0 mL distilled water.

The expected values are in the concentration range indicated on the QC datasheet.

It is recommended to store controls after reconstitution at -20 °C.

WASH | SOLN | 20x Wash solution, 20x concentrated:

1 vials, each contains 50 mL wash-concentrate

Dilute wash-concentrate solution before use up to 1.0 L with distilled water.

The diluted washing-solution is stable at 4 °C up to the expiry date of the kit.

Note: Temperatures and expiry dates printed on component vial labels, apply to the long-term storage by manufacturer only, prior to assembling of the kit. Do not take into account.

5 MATERIAL REQUIRED BUT NOT PROVIDED

In addition to standard laboratory equipment, the following items are required:

- precision micropipets (100 µL)
- semi-automatic pipets (100 µL & 2.0 mL)
- vortex type mixer
- horizontal or orbital shaker
- aspiration system
- gamma counter set for 125 iodine

6 SPECIMEN COLLECTION AND STORAGE

- Collect blood in tubes containing or not additives.
- Separate serum or plasma from cells by centrifugation.
- Serum and plasma samples may be stored at 2 °C - 8 °C, if the assay is to be performed within 24 hours.
  For longer storage keep frozen (at < -18 °C) after aliquoting so as to avoid repeated freezing and thawing. If samples have concentrations greater than the highest standard, they must be diluted into the zero standard

7 ASSAY PROCEDURE

7.1 Preparation of reagents

Let all the reagents come to room temperature.

- Each content of the **control** is reconstituted with the volume of distilled water indicated on the label (1.0 mL. Wait for 30 min following reconstitution and mix gently to avoid foaming before dispensing.
  Store reconstituted solutions at 2 °C - 8 °C until the expiry date of the kit or at -20 °C for a longer time.
- **Preparation of the wash solution**
  Pour the content of the vial into 1.0 L of distilled water and homogenize. The diluted solution may be stored at 2 °C - 8 °C until the expiry date of the kit.

7.2 Procedure

Allow reagents and samples to warm up at room temperature.

Mix reagents by gentle agitation before use.

1. Prepare coated-tubes for:
   Standards, Controls and Samples and Recoveries in duplicate.
   Uncoated test-tubes may be used for the Total Activity.
2. Pipette 100 µL of each Standard, Control and Sample into the corresponding coated tubes.
3. Add 100 µL of I-125-TSH-Ab-Tracer into all tubes, also for the Total-Activity.
4. Mix the tubes on vortex and incubate for 60 minutes at room temperature on an orbital shaker (min.300 - 350 rpm).
5. Perform an aspiration and washing cycle, with 2 mL of diluted washing solution on all tubes, except those for Total Activity
6. Repeat the aspiration and washing cycle, in all twice.
7. Aspirate or decant completely the contents or liquid of the tubes
8. Count the radioactivity in all tubes for 1 minute by using a gamma-counter.

8 RESULTS

Results are obtained from the standard curve by interpolation. The curve serves for the determination of TSH concentrations in samples measured at the same time as the standard.

8.1 Standard curve

The results in the package insert were calculated using a linear curve fit with B/T (%) or B/Bmax (%) on vertical axis and the TSH concentration of the standards on the horizontal axis (mIU/L).

Other data reduction methods may give slightly different results.

Results of a typical assay

Do not use the data listed below in place of standard-curve determined at the time of the assay.

Totalaktivität : 193875 cpm				
Standard	mIU/L	cpm (n=3)	B/ T (%)	B/B_{max} (%)
0	0	48	0.02	0.08
1	0.15	301	0.16	0.50
2	0.50	882	0.46	1.46
3	1.5	2 456	1.27	4.06
4	5.0	7 799	4.02	12.9
5	15.0	21 889	11.3	36.2
6	50.0	60 513	31.2	100

Samples

Locate for each sample the B/T (%) or the B/B0 (%) on the vertical axis and read off the corresponding the TSH concentration of the sample on the horizontal axis in mIU/L.

9 QUALITY CONTROL

Good laboratory practices imply that control samples be used regularly to ensure the quality of the results obtained. These samples must be processed exactly in the same way as the assay samples, and it is recommended that their results be analysed using appropriate statistical methods.

10 EXPECTED VALUES

It is recommended, that each laboratory establishes its own normal values. The following values obtained with healthy subjects are indicative only.

Euthyroid :	0.2 - 4.0 mIU/L
Hyperthyroid:	≤ 0.15 mIU/L
Untreated hypothyroid:	> 5.0 mIU/L

11 PERFORMANCE CHARACTERISTICS

(for more details, see the data sheet "APPENDIX")

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

11.1 Sensitivity

Analytical sensitivity: 0.04 mIU/L

Functional sensitivity: 0.141 mIU/L

11.2 Specificity

The antibody used in the immunoassay is highly specific for TSH. Extremely low cross reactivities were obtained against several related molecules (LH, FSH, hCG, GH, Prolactin).

11.3 Precision

Intra-assay

Samples were assayed in 10 times in the same series. The coefficients of variation were found below or equal to 3.7 % for serum samples.

Inter-assay

Samples were assayed in duplicate in 16 different series. The coefficients of variation were found below or equal to 8.6 % for serum samples.

11.4 Accuracy

Dilution test

High-concentration samples were serially diluted with the zero calibrator. The recovery percentages obtained were between 92.7 % and 109 %.

Recovery test

Low-concentration samples were spiked with known quantities of TSH. The recovery percentages obtained were between 99.4 % and 107 %.

11.5 Measurement range

(from analytical sensitivity to highest calibrator):

0.04 to approximately 50 mIU/L.

11.6 LIMITATIONS

The non-respect of the instructions in this package insert may affect results significantly. Results should be interpreted in the light of the total clinical presentation of the patient, including clinical history, data from additional tests and other appropriate information. Do not use hemolyzed, lipemic or icteric samples. Shortage of incubation time to 30 minutes was tested on SR300 instrument. Performance characteristics of the assay are not guaranteed if different automate is used.

For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

Short instruction

			NSB	Standard	Controls	Samples	Total activity
Pipette:	Standard 0	µL	100				
	Standard 1 - 6 ,	µL		100			
	Controls 1 & 2	µL			100		
	Samples	µL				100	
Pipette:	Tracer I-125	µL	100	100	100	100	100
Incubate:	60 Min at RT (min. 300 – 350 rpm) shaking						
Decant:	Aspirate or Decant						
Wash:	Wash-Solution	mL	2.0	2.0	2.0	2.0	
Decant:	Aspirate or decant Wash tubes twice with 2.0 mL						
Measure:	Recommended measuring time: 1 Minute						

12 APPENDIX - PERFORMANCE CHARACTERISTICS

Representative data are provided for illustration only. Performance obtained in individual laboratories may vary.

12.1 Specificity

Data on cross-reactivity with several hormones are presented in the following table: Cross-reactivity (%) = TSH concentration *(for B/Bmax= 0.5)* x 100/ Hormone concentration (*for B/Bmax= 0.5)*

Hormone	Cross-reactivity (%)
TSH	100
LH	ND
FSH	ND
hCG	ND
GH	ND
Prolactin	ND

ND = Non-Detectable (<0.1 %)

12.2 Precision

Intra-assay

Serum samples	**S1**	**S2**	**S3**	**S4**
Number of determinations	10	10	10	10
Mean value (mIU/L)	2.02	5.83	10.0	42.0
CV (%)	3.0	2.5	3.2	3.7

EDTA plasma samples	**P1**	**P2**	**P3**
Number of determinations	25	25	25
Mean value (mIU/L)	1.15	8.30	31.32
CV (%)	3.47	1.88	2.77

Inter-assay

Serum samples	**S1**	**S2**	**S3**
Number of determinations	16	16	16
Mean value (mIU/L)	3.10	9.7	39.6
CV (%)	8.6	5.7	2.8

EDTA plasma samples	**P1**	**P2**	**P3**
Number of determinations	10	10	10
Mean value (mIU/L)	1.52	18.5	33.4
CV (%)	3.36	4.36	1.76

12.3 Accuracy

Dilution test

Serum samples were diluted in zero calibrator and assayed according to the assay procedure of the kit.

Serum	Dilution factor	TSH (mIU/L)		Ratio (%) Measured/ Expected
		Measured	Expected	
S1	undil.	47.9	-	-
	1/2	22.4	23.95	93.5
	1/4	11.1	11.98	92.7
	1/8	5.8	5.99	96.9
	1/16	3.0	3.0	100.0
S2	undil.	18.3	-	-
	1/2	9.3	9.15	101.6
	1/4	4.6	4.58	100.5
	1/8	2.5	2.29	109.3
	1/16	1.2	1.14	105.0
S3	undil.	49.7	-	-
	1/2	24.5	24.85	98.6
	1/4	12.5	12.43	100.6
	1/8	6.1	6.21	98.2
	1/16	3.2	3.11	103.0

EDTA plasma	Dilution factor	TSH (mIU/L)		Ratio (%) Measured/ Expected
		Measured	Expected	
P1	undil.	6.23	-	-
	1/2	3.10	3.12	99.52
	1/4	1.63	1.56	104.7
	1/8	0.75	0.78	96.31
	1/16	0.40	0.39	102.7
	1/32	0.22	0.19	113.0
P2	undil.	7.81	-	-
	1/2	3.99	3.91	102.2
	1/4	1.93	1.95	98.85
	1/8	0.98	0.98	100.4
	1/16	0.49	0.49	100.4
	1/32	0.24	0.24	98.34
P3	undil.	7.53	-	-
	1/2	3.78	3.77	100.4
	1/4	1.85	1.88	98.27
	1/8	1.00	0.94	106.2
	1/16	0.45	0.47	95.62
	1/32	0.22	0.24	93.49
P4	undil.	8.86	-	-
	1/2	4.48	4.43	101.1
	1/4	2.22	2.22	100.2
	1/8	1.13	1.11	102.0
	1/16	0.56	0.55	101.1
	1/32	0.32	0.28	115.6
P5	undil.	6.77	-	-
	1/2	3.32	3.39	98.08
	1/4	1.62	1.69	95.72
	1/8	0.83	0.85	98.08
	1/16	0.43	0.42	101.6
	1/32	0.24	0.21	113.4

Plasma samples were diluted in the zero calibrator and assayed according to the procedure of the kit.

12.4 Recovery test

TSH was added to 5 serum samples and assayed according to the procedure of the kit.

Serum	Dilution factor	TSH (mIU/L)		Ratio (%) Measured/ Expected
		Measured	Expected	
S1	undil.	47.9	-	-
	1/2	22.4	23.95	93.5
	1/4	11.1	11.98	92.7
	1/8	5.8	5.99	96.9
	1/16	3.0	3.0	100.0
S2	undil.	18.3	-	-
	1/2	9.3	9.15	101.6
	1/4	4.6	4.58	100.5
	1/8	2.5	2.29	109.3
	1/16	1.2	1.14	105.0
S3	undil.	49.7	-	-
	1/2	24.5	24.85	98.6
	1/4	12.5	12.43	100.6
	1/8	6.1	6.21	98.2
	1/16	3.2	3.11	103.0

TSH was added to 5 EDTA-plasma samples and assayed according to the procedure of the kit.

EDTA plasma	Endogen. conc. (mIU/L)	Added conc. (mIU/L)	Expected conc. (mIU/L)	Measured conc. (mIU/L)	Ratio (%) Measured/ Expected
P1	3.43	1.22	4.65	4.60	98.85
	3.35	2.38	5.73	5.63	98.20
	3.20	4.55	7.75	7.43	95.93
P2	3.07	1.22	4.29	4.33	100.9
	3.00	2.38	5.38	5.57	103.5
	2.86	4.55	7.41	7.42	100.2
P3	1.70	1.22	2.92	2.87	98.39
	1.66	2.38	4.04	4.09	101.3
	1.58	4.55	6.13	6.03	98.41
P4	4.75	1.22	5.97	5.94	99.49
	4.64	2.38	7.02	7.21	102.7
	4.43	4.55	8.97	9.11	101.5
P5	1.25	1.22	2.47	2.55	103.3
	1.22	2.38	3.60	3.62	100.6
	1.16	4.55	5.71	6.01	105.3

^{125}I Characteristics

T1/2 (125I) = 1443 h = 60.14 d

125I	E (MeV)	%
γ	0.035	
X	0.027	114
	0.032	25

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SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
	Caution *	Achtung *			
RUO	For research use only	Nur für Forschungszwecke	Solo a scopo di ricerca	Sólo para uso en investigación	Seulement dans le cadre de recherches
Distributed by	Distributed by	Vertreiber	Distributore	Distribuidor	Distributeur
Content	Content	Inhalt	Contenuto	Contenido	Contenu
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité