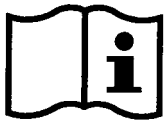
Product information

Information about other products is available at: www.demeditec.com



Useŕs Manual

Thyroglobulin (hTg) IRMA

Immunoradiometric assay for the direct quantitative determination of human thyroglobulin (hTg) in human serum

CE

IVD

REF **DE20100**

Σ **100 tubes**

Demeditec Diagnostics GmbH • Lise-Meitner-Straße 2 • 24145 Kiel (Germany)
Phone: +49 (0)431/71922-0 • Fax. +49 (0)431/71922-55
info@demeditec.de • www.demeditec.com

Version ACE190831
DAR
Updated 191114

The ^{125}I-hTg IRMA system provides direct quantitative *in vitro* determination of human thyroglobulin (hTg) in human serum. hTg can be assayed in the range of 0-250 ng/ml using 100 µl serum samples.

Introduction

The Thyroglobulin is a iodoglycoprotein consisting of heterogeneous molecules, the composition of which is in part depending on the degree of iodination. The prevailing molecular form is 660 kDa (dimeric form, the two subunits, linked by noncovalent bounds), but both larger and smaller molecular forms exist in the thyroid gland. Tg is the site of synthesis and storage of thyroid hormones produced by the thyroid gland. Tg is synthesized and stored in thyroid follicles and some of the nonenzimatically digested protein is released into the circulation upon stimulation with thyrotropin (TSH) together with thyroxin (T4) and triiodothyronin (T3).

The determination of Tg by immunoassay methods plays a crucial role in the diagnosis of thyroid disorders, such as Grave's multinodular goiter, benign thyroid adenoma, thyroiditis (acute phase), and differentiated carcinoma, and is a useful tool in monitoring patients at risk for thyroid carcinoma after previous irradiation.

The sensitivity of the present hTg IRMA system makes it suitable for the measurement of subnormal hTg levels, which is an early and reliable marker of tumor recurrence.

Principle of method

The technology uses two high affinity monoclonal antibodies in an immunoradiometric assay (IRMA) system.

The ^{125}I labelled signal-antibody binds to an epitope of the Tg molecule spatially different from that recognised by the biotin-capture-antibody. The two antibodies react simultaneously with the antigen present in standards or samples, which leads to the formation of a capture antibody - antigen - signal antibody complex, also referred to as a "sandwich".

During an overnight incubation period the immuno-complex is immobilized to the reactive surface of streptavidin coated test tubes. Reaction mixture is then discarded, test tubes washed exhaustively, and the radioactivity is measured in a gamma counter.

The concentration of antigen is directly proportional to the radioactivity measured in test tubes. By constructing a calibration curve plotting binding values against a series of calibrators containing known amount of hTg, the unknown concentration of hTg in patient samples can determined.

Contents of the kit

1. **Anti-hTG** **I-125** 1 bottle TRACER (21 ml), ready to use, containing about 980 kBq ^{125}I-anti-hTg and capture anti-hTg in buffer with red dye 0.1 % NaN3.
2. **CAL** **1** **-** **6** 6 vials STANDARD (6 x 1.0 ml), containing (S1-S6) 0.3, 1.0, 4.0, 20, 100, 250 ng/ml hTg (calibrated to BCR CRM457) in serum with 0.1% NaN_3.
3. **CONTROL** **1** / **2** 2 vials CONTROL SERUM, with 0.1% NaN_3. The concentration of the control sera is specified in the quality certificate enclosed.
4. **DIL** SERUM DILUENT, 5.0 ml, containing 0.1% NaN_3
5. **RECOV** RECOVERY SERUM, 1.0 ml, containing 0.1% NaN_3 The concentration of the recovery serum is specified in the quality certificate enclosed.
6. **SORB** **CT** 2 boxes COATED TUBE, Ready to use. 2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.
7. **WASH** **SOLN** **35x** 1 bottle WASH BUFFER CONCENTRATE (20 ml), containing 0.2 % NaN_3. See *Preparation of reagents.*

Quality certificate

Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (100, 200 and 2000 µl), vortex mixer, shaker, plastic foil, adsorbent tissue, gamma counter

Recommended tools and equipment

repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L reservoir (instead of the 2-ml pipette)

Specimen collection and storage
Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Samples can be stored at 2-8 °C if the assay is carried out within 24 hours, otherwise aliquots should be prepared and stored deep frozen (-20°C). Frozen samples should be thawed and thoroughly mixed before assaying. Repeated freezing and thawing should be avoided. Do not use lipemic, hemolyzed or turbid specimens. Samples with a hTg concentration higher than that of the most concentrated standard should be diluted and reassayed.

Preparation of reagents, storage
Add the wash buffer concentrate (20 ml) to 700 ml distilled water to obtain 720 ml wash solution. Upon dilution store at 2-8°C until expiry date.
Store the rest of reagents between 2-8°C after opening. At this temperature each reagent is stable until expiry date. The actual expiry date is given on the package label and in the quality certificate.
CAUTION!
Equilibrate all reagents and serum samples to room temperature. Mix all reagents and samples thoroughly before use. Avoid excessive foaming.

Recovery test:
Anti-Tg antibodies or unspecific effects in a patient's serum can interfere with serum thyroglobulin measurement, which leads to underestimation of the Tg concentration in IRMA system. Interference can be detected by using recovery test. The recovery test should be carried out as described in the assay procedure.
The concentration of the recovery serum (approximately 500 ng Tg/ml) should be checked with serum diluent (recovery reference tubes (DR)).
Recovery (in%) in the serum sample:

$$\frac{\text{ng Tg/ml } R_x - \text{ng Tg/ml } S_x}{\text{ng Tg/ml DR}} \text{ x100 = \% recovery}$$

Recoveries between 70% and 130% are considered valid. Levels of <70% or >130% are due to interference and the Tg level of the relevant original sample is considered invalid.

Assay procedure
(For a quick guide , refer to Table 1.)
1. Equilibrate reagents and samples to room temperature before use.
2. Label coated tubes in duplicate for each standard (S1-S6), control serum (CI, CII), serum diluent (D) as zero calibrator, recovery reference (DR), serum samples (S_x), and recovery serum (R_x).
3. Homogenize all reagents and samples by gentle mixing. Avoid foaming.
4. Pipette 10µl recovery serum into the recovery reference tubes (DR) and into the sample recovery tubes (R).
5. Pipette 100 µl of standards into the standard tubes (S1-S6), 100 µl control into control tubes (CI, CII), 100µl sample into sample (S) and recovery tubes (R) and 100µl serum diluent into the recovery reference tubes (DR) and serum diluent tubes (D) as zero calibrator. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
6. Pipette 200 µl of tracer into each tube.
7. Gently vortex all tubes. Seal all tubes with a plastic foil.
8. Incubate tubes for 15-24 hours at RT (room temperature).
9. Add 2,0 ml diluted wash buffer to each tube and decant the supernatant from all tubes by the inversion of the rack. In the upside-down position, place the rack on an absorbent paper for 2 minutes.
10. Return the tube-rack to an upright position, and repeat Step-9 two times more
11. Count each tube for at least 60 seconds in a gamma counter.
12. Calculate the Tg concentrations of the samples as described in calculation of results or use special software.

Table 1. Assay Protocol, Pipetting Guide (all volumes in microlitres)

Tube Reagents	Total (T)	Serum diluent (D)	Standard (S_1-S_6)	Sample (Sx)	Recovery tubes (Rx)	Recovery reference tubes (D_R)	Control serum (CI-CII)
Standard			100				
Sample				100	100		
Control serum							100
Recovery serum					10	10	
Serum diluent		100				100	
Tracer	200	200	200	200	200	200	200
Incubate tubes for 15-24 hours at RT							
Wash buffer		2000	2000	2000	2000	2000	2000
Decant the fluid and blot on filter paper							
Repeat the washing step two times							
Counting radioactivity (60 sec/tube)							
Calculation							

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in Table 2.

Calculate the average count per minute (CPM) for each pair of assay tubes

Calculate the normalized percent binding for each standard, control and sample respectively by using the following equation:

$$B/T(\%) = \frac{S_{1\text{-}6} / C / Sx / Rx\ (cpm) - D}{T(cpm)} \times 100$$

Using semi-logarithmic graph paper plot B/T (%) for each standard versus the corresponding concentration of Tg.

Determine the Tg concentration of the unknown samples by interpolation from the standard curve. Do not extrapolate values beyond the standard curve range.

Out of fitting programs applied for computerized data processing logit-log, or spline fittings can be used.

Automated data processing systems are also available.

Table 2. Typical assay data

Tubes	Tg ng/ml	Count cpm 1	Count cpm 2	Mean cpm	Tg ng/ml
T	-	393128	394123	393626	-
D (NSB)	0	167	178	171	
$S_{0.3}$	0.3	552	559	556	-
$S_{1,0}$	1.0	1448	1476	1462	-
$S_{4,0}$	4.0	5517	5500	5509	-
S_{20}	20	24756	24712	24734	-
S_{100}	100	90031	90207	90119	-
S_{250}	250	158821	162358	160590	-
CI	-	2458	2502	2480	1.99
CII	-	70250	70511	70380	69.8

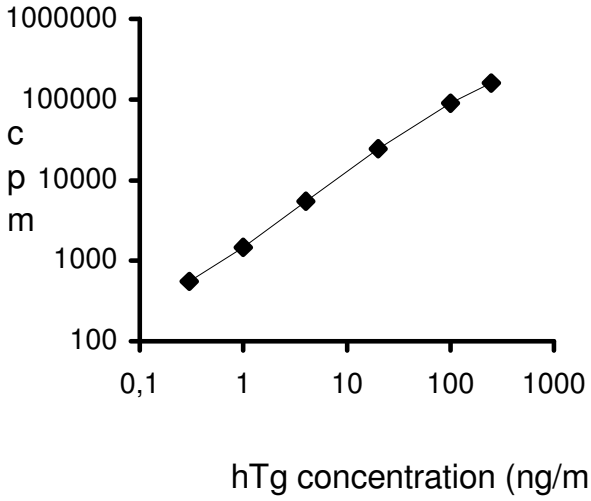


Figure 1: A typical standard curve
(*Do not use to calculate unknown samples)*

Characterization of assay

Sensitivity

The analytical sensitivity or minimum detectable limit is calculated by the interpolation of the mean counts of zero standard plus 2 standard deviation from the standard curve. Determination was carried out using 20 replicates of zero standard response.

The value of analytical sensitivity is **0.03** ng/ml measured using fresh tracer and **0.09** ng/mL using a tracer close to the expiry date.

The functional sensitivity was determined based on the precision profile obtained from 20 independent assay runs of low concentration samples in duplicate. The value of functional sensitivity is **0.1** ng/ml measured using fresh tracer and **0.3** ng/mL using a tracer close to the expiry date.

Hook effect

There is no high dose hook effect up to an hTg concentration of 20000 ng/ml.

Linearity – dilution test

Three individual human serum samples were diluted with the zero standard of the KIT. The diluted samples were measured according to KIT protocol.

sample No.	dilution factor	expected ng/ml	observed ng/ml	recovery %
1		68.5	68.5	
1	2	34.0	34.3	101.0
1	4	16.7	17.0	101.6
1	8	8.2	8.3	101.0
1	16	4.1	4.2	104.2
2		93.6	93.6	
2	2	46.4	45.2	97.5
2	4	22.9	22.0	96.3
2	8	11.3	10.7	95.2
2	16	5.6	5.3	95.2
3		67.5	67.5	
3	2	33.6	31.8	94.7
3	4	16.6	15.4	93.0
3	8	8.2	7.5	92.3
3	16	4.0	3.7	93.0

Recovery – addition test

49 individual human serum samples were spiked with known concentration serum based stock solution made from BCR certification reference preparation 457 in different amount. The results are summarised below.

	sample base concentration (ng/ml)	**concentration with Recovery sample (ng/ml)**	**Recovery %**
Sample 1	6.9	60.6	101
Sample 2	15.5	69.9	103
Sample 3	99.5	152.6	100
Sample 4	2.3	58.8	107
Sample 5	10.4	63.5	100
Sample 6	57.3	109.0	97
Sample 7	6.5	62.0	105
Sample 8	7.0	61.0	102
Sample 9	2.5	52.8	95
Sample 10	106.0	159.4	101
Sample 11	32.4	83.8	97
Sample 12	7.4	60.8	101
Sample 13	41.0	88.2	89
Sample 14	8.8	63.9	104
Sample 15	0.9	56.9	106
Sample 16	12.9	61.0	91
Sample 17	0.3	51.1	96
Sample 18	6.2	61.3	104
Sample 19	3.9	58.7	103
Sample 20	2.0	51.6	94
Sample 21	3.4	52.2	92
Sample 22	3.3	55.7	99
Sample 23	43.9	91.8	90
Sample 24	10.1	62.7	105
Sample 25	8.6	62.9	109
Sample 26	0.6	36.8	72
Sample 27	0.3	54.8	109
Sample 28	11.4	52.3	82
Sample 29	10.3	63.8	107
Sample 30	7.9	64.5	113
Sample 31	0.6	57.2	113
Sample 32	33.3	76.3	86
Sample 33	5.0	64.8	120
Sample 34	11.5	68.3	114
Sample 35	22.9	71.0	96
Sample 36	2.3	50.4	96
Sample 37	7.9	53.9	92
Sample 38	0.5	39.5	78
Sample 39	5.4	48.2	86
Sample 40	17.2	73.7	113
Sample 41	190.8	235.4	89
Sample 42	77.5	132.1	109
Sample 43	5.1	61.5	113
Sample 44	23.1	66.2	107
Sample 45	76.4	119.4	107
Sample 46	4.7	47.2	106
Sample 47	154.2	199.1	112
Sample 48	2.3	44.2	104

Intra-inter-assay

Intra-assay		Inter-assay	
mean (ng/ml)	CV %	mean (ng/ml)	CV %
11,4	1,8	11,5	2,2
118,7	1,9	117,8	2,7
85,4	2,2	86,2	1,9
5,9	2,6	6,0	1,7
4,9	2,0	5,1	2,0
1,3	2,7	1,3	3,0
0,7	5,9	0,7	6,3

Expected Values

Expected normal value range is 2 ng/ml - 70 ng/ml.
It is recommended that each laboratory determine a reference range for healthy persons for its own patient population, since this may vary in different laboratories or regions

Procedural notes

1) **Source of error!** Reactive test tubes packed in plastic boxes are not marked individually. Care should be taken of not mixing them with common test tubes. To minimize this risk, never take more tubes than needed out of plastic box, and put those left after work back to the box. It is recommended to label assay tubes by a marker pen.

2) **Source of error!** To ensure the efficient rotation, tubes should be firmed tightly inside the test tube rack. Never use a rack type with open hole. An uneven or incomplete shaking may result in a poor assay performance.

3) **Addition of wash buffer.** For the addition of wash buffer the use of a common laboratory dispenser equipped with a 1-L glass bottle, and a flexible outlet tubing end is recommended. In lack of this tool a large-volume syringe attached to a repeating pipette can be used.

Additional information

Components from various lots or from kits of different manufacturers should not be mixed or interchanged.

Precaution

Radioactivity

This product contains radioactive material. It is the responsibility of the user to ensure that local regulations or code of practice related to the handling of radioactive materials are satisfied.

Biohazard

Human blood products used in the kit have been obtained from healthy human donors. They were tested individually by using approved methods (EIA, enzyme immunoassay), and were found to be negative, for the presence of both Human Immunodeficiency Virus antibody (Anti-HIV-1, 2), Hepatitis-C antibody (anti-HCV), Hepatitis B surface Antigen (HBsAg) and Treponema Antibody.
Care should always be taken when handling human specimens to be tested with diagnostic kits. Even if the subject has been tested, no method can offer complete assurance that infectious agents are absent. Human blood samples should therefore be handled as *potentially infectious materials*.
All animal products and derivatives have been collected from healthy animals. Nevertheless, components containing animal substances should be treated as *potentially infectious materials*.

Chemical hazard

Components contain sodium azide as an antimicrobial agent. Dispose of waste by flushing with copious amount of water to avoid build-up of explosive metallic azides in copper and lead plumbing. The total azide present in each pack is 76 mg.

Storage and shelf life

Store this product at a temperature of 2-8°C
Shelf-life: 67 days from availability.

Literature

1: Feldt-Rasmussen U et al. Ann Biol Clin (Paris). 1996;54(10-11):337-42. Human thyroglobulin reference material (CRM 457). 1st Part: Assessment of homogeneity, stability and immunoreactivity.
2: Feldt-Rasmussen U et al. Ann Biol Clin (Paris). 1996;54(10-11):343-8. Human thyroglobulin reference material (CRM 457). 2nd Part: Physicochemical characterization and certification.
3: Van Herle AJ, Van Herle IS, Greipel MA. J Clin Endocrinol Metab. 1985 Feb;60(2):338-43. An international cooperative study evaluating serum thyroglobulin standards.
4: Feldt-Rasmussen U, Schlumberger M.. J Endocrinol Invest. 1988 Mar;11(3):175-81. European interlaboratory comparison of serum thyroglobulin measurement.
5: Spencer CA, Platler BW, Nicoloff JT. Clin Chim Acta. 1985 Dec 13;153(2):105-15. The effect of [125I]thyroglobulin tracer heterogeneity on serum Tg RIA measurement.
6: Ferrari L et al. Q J Nucl Med Mol Imaging. 2004 Sep;48(3):237-42. Comparative evaluation of two methods to assay thyroglobulin serum concentrations in patients with differentiated thyroid arcinomas.
7: Spencer CA et al. J Clin Endocrinol Metab. 2005 Oct;90(10):5566-75. Epub 2005 Jun 28. Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas.
8: Morgenthaler NG et al. Clin Chem. 2002 Jul;48(7):1077-83. Technical evaluation of a new immunoradiometric and a new immunoluminometric assay for thyroglobulin.

SYMBOLS USED WITH DEMEDITEC ASSAYS

Symbol	English	Deutsch	Francais	Espanol	Italiano
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conforme aux normes européennes	Conformidad europea	Conformità europea
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las Instrucciones	Consultare le istruzioni per l'uso
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Ussage Diagnostic in vitro	Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Référence	Número de catálogo	No. di Cat.
LOT	Lot. No. / Batch code	Chargen-Nr.	No. de lot	Número de lote	Lotto no
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Note warnings and precautions	Warnhinweise und Vorsichtsmaßnahmen beachten	Avertissements et mesures de précaution font attention	Tiene en cuenta advertencias y precauciones	Annoti avvisi e le precauzioni
	Storage Temperature	Lagerungstemperatur	Temperature de conservation	Temperatura de conservacion	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributtore