Instructions for Use

Total T4 RIA

IVD

CE

REF **RIA-4524**

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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.

Table of Contents

Radioimmunoassay for the in vitro determination of total thyroxine (TT4) in human serum and plasma.
For *in vitro* diagnostic use.

1 PRINCIPLE OF THE ASSAY

The radioimmunoassay of total thyroxine (TT4) is a competition assay.

Samples and calibrators are incubated with ^{125}I-labeled T4, as tracer, in antibody-coated tubes. After incubation, the liquid content of tubes is aspirated and the bound radioactivity is determined in a gamma counter. A standard curve is constructed and unknown values are obtained from the curve by interpolation.

2 WARNINGS AND PRECAUTIONS

2.1 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 established with each assay.
- It is recommended to perform the assay in duplicate.
- Each tube must be used only once.

2.2 Basic rules of radiation safety

The purchase, possession, utilization, and transfer of radioactive material are 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.

2.3 Sodium azide

Some reagents contain sodium azide as a preservative. Sodium azide can 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.

2.4 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. Waste should be discarded according to the country rules.

2.5 GHS HAZARD CLASSIFICATION

Not classified as hazardous.

Safety Data Sheet is available upon request.

3 SPECIMEN COLLECTION, PROCESSING, STORAGE AND DILUTION

Collect blood in dry tubes or in tubes with EDTA.

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 (up to 1 month) keep frozen at < -20 °C after aliquoting so as to avoid repeated freezing and thawing. Thawing of sample should be performed at room temperature.

Serum and EDTA plasma values for 15 samples (serum values ranging from 71.08 to 167.32 nmol/L) were compared using the RIA-4524 Total T4 RIA kit. Results are as follows:

[EDTA-plasma] = 0.6673[serum]+22.12

R = 0.9749

4 MATERIALS PROVIDED

All reagents of the kit are stable until the expiry date indicated on the kit label, if stored at 2 °C - 8 °C. Expiry dates printed on vial labels apply to the long-term storage of components by the manufacturer only, prior to assembly of the kit. Do not take into account.

Storage conditions for reagents after reconstitution are indicated in paragraph Assay Procedure.

1. **Anti-T4 monoclonal antibody-coated tubes:**
   2 x 50 tubes (ready-to-use)
2. **^{125}I-labeled T4 tracer:**
   one 55 mL vial (ready-to-use)
   The vial contains 110 kBq, at the date of manufacture, of ^{125}I-labeled T4 in buffer with proteins, sodium azide (<0.1%) and a dye.
3. **Calibrators:**
   six 0.5 mL vials (ready-to-use)
   The calibrator vials contain from 0 to approximately 400 nmol/L of T4 in human serum with sodium azide (<0.1%).
   The exact concentration is indicated on each vial label.
   The calibrators were calibrated using the standard IRMM-468.
4. **Control serum:**
   two vials (lyophilised)
   The vials contain T4 lyophilised in human serum with sodium azide (<0.1%).
   The expected values are in the concentration range indicated on the supplement.

5 MATERIAL REQUIRED, BUT NOT PROVIDED

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

- Precision micropipets (20 µL).
- Semi-automatic pipets (500 µL).
- Vortex type mixer.
- Horizontal or orbital shaker.
- Aspiration system.
- Gamma counter set for 125 iodine.

6 RESULTS

Results are obtained from the standard curve by interpolation. The curve serves for the determination of TT4 concentrations in samples measured at the same time as the calibrators.

6.1 Standard curve

The results in the quality control department were calculated using weighted cubic regression curve fit with B/T or B/B_0 on the logit vertical axis and analyte concentration of the calibrators on the log horizontal axis (nmol/L). Other data reduction methods may give slightly different results.

Total activity: cpm 44,140				
Calibrators	**TT4 (nmol/L)**	**cpm (n=3)**	**B/T (%)**	**B/B_0 (%)**
0	0	34,959	77.2	100
1	26	31,590	71.6	90.4
2	53	26,213	59.4	75.0
3	105	17,048	38.6	48.8
4	210	9,727	22.0	27.8
5	420	5,187	11.8	14.8

(Example of standard curve, do not use for calculation)

6.2 Samples

Locate for each sample the ratio B/T or B/B_0 on the vertical axis of the standard curve and read-off the corresponding TT4 concentration of the sample on the horizontal axis in nmol/L.

To convert concentrations from nmol/L to ng/dL, multiply results by 77.7.

7 EXPECTED VALUES

It is suggested that each laboratory establishes its own normal values.

Normal concentration range of total T4 in serum for untreated euthyroid individuals was found:

	N	**Mean, (nmol/L)**	**Median, (nmol/L)**	**Min - Max, (nmol/L)**	**2.5^{th} - 97.5^{th} percentile (nmol/L)**
Female	50	118.3	114.7	59.65 - 168.2	74.1 - 160.3
Male	50	96.42	96.76	59.13 - 141.4	68.91 - 122.3
Male and Female	100	107.4	101.9	59.13 - 168.2	69.32 - 159.7

8 QUALITY CONTROL

Good laboratory practices imply that control samples must 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 to analyze their results using appropriate statistical methods.

In case of packaging deterioration or if data obtained show some performance alteration, please contact your local distributor or use the following e-mail address: drg@drg-diagnostics.de

9 PROCEDURE

9.1 Reconstitution of control serum

The contents of the vials must be brought to room temperature before reconstitution with the volume of distilled water indicated on the vial label.

Wait for 10 min following reconstitution and mix gently to avoid foaming before dispensing.

Store the reconstituted solutions at 2 °C - 8 °C for one week or aliquoted at < -18 °C until the expiry date of the kit.

9.2 Assay procedure

Bring all reagents to room temperature before pipeting.

9.3 Assay procedure

Step 1 Additions *	Step 2 Incubation	Step 3 Counting
To antibody coated tubes, add successively: - 20 µL of calibrator, control or sample and - 500 µL of tracer. Mix.	Incubate 1 hour at 18 °C - 25 °C with shaking (> 280 rpm).	Aspirate carefully the content of tubes (except the 2 tubes «total cpm»). Count bound cpm (B) and total cpm (T) for 1 min.

* Add 500 µL of tracer to 2 additional tubes to obtain total cpm.

10 PERFORMANCE CHARACTERISTICS

(For more details, see the data sheet “APPENDIX”)

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

10.1 Sensitivity

10.1.1 Analytical sensitivity: 10.63 nmol/L

10.1.2 Functional sensitivity: 16.71 nmol/L

10.2 Specificity

The antibody used in the immunoassay is highly specific for T4. Extremely low cross reactivities were obtained with several related molecules (e.g. L-T3).

10.3 Precision

10.3.1 Intra-assay

Samples were assayed 25 times in the same series.

The coefficients of variation were found below or equal to 3.29 %.

10.3.2 Inter-assay

Samples were assayed in duplicate in 10 different series.

The coefficients of variation were found below or equal to 7.53 %.

10.4 Accuracy

10.4.1 Dilution test

High-concentration samples were serially diluted with the zero calibrator. The recovery percentages obtained were between 88.1 % and 112 % for serum.

10.4.2 Recovery test

Samples were spiked with known quantities of TT4. The recovery percentages were obtained between 81.0 % and 107 % for serum.

10.5 Measurement range

(from analytical sensitivity to highest calibrator):

10.63 to approximately 400 nM..

11 LIMITATION OF THE METHOD

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.

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.

12 APPENDIX

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:

Analogue	Cross reaction (%)
L-T4, (L-thyroxine), (3,3,5,5-tetraiodo-L-thyronine)	100
L-T3 - (3,3,5-triiodo-L-thyronine)	ND
3,5-diiodo-L-thyronine ND	ND
3,5-diiodo-L-thyrosine dihydrate	ND
3,3,5-triiodothyroacetic acid (TRIAC)	ND

ND = Non-Detectable

12.2 Precision

12.2.1 Intra-assay

Serum	S1	S2	S3
Number of determinations	25	25	25
Mean (nmol/L)	89.35	122.0	265.2
CV (%)	3.29	2.64	2.55

Plasma-EDTA	P1	P2	P3
Number of determinations	25	25	25
Mean (nmol/L)	58.52	271.8	389.6
CV (%)	6.45	5.18	5.97

12.2.2 Inter-assay

Serum	S1	S2	S3
Number of determinations	10	10	10
Mean (nmol/L)	53.23	115.6	457.4
CV (%)	6.82	4.67	7.53

Plasma-EDTA	P1	P2	P3
Number of determinations	10	10	10
Mean (nmol/L)	40.78	117.3	353.6
CV (%)	10.70	8.68	4.74

12.3 Accuracy

12.3.1 Dilution test

Five serum and plasma samples were chosen for dilution test. Samples were serially diluted by calibrator 0 and assayed. Results are shown below:

		Serum		
Serum	**Dilution**	**Measured (nmol/L)**	**Expected (nmol/L)**	**Ratio (%) Measured/ Expected**
S1	-	166.1	-	-
	1:2	86.84	83.03	104.6
	1:4	36.58	41.51	88.12
	1:8	21.17	20.76	102.0
S2	-	234.0	-	-
	1:2	116.6	117.00	99.69
	1:4	57.55	58.50	98.38
	1:8	28.47	29.25	97.34
	1:16	13.60	14.62	93.00
S3	-	257.1	-	-
	1:2	132.2	128.54	102.8
	1:4	65.56	64.27	102.0
	1:8	34.09	32.14	106.1
	1:16	15.87	16.07	101.5
S4	-	253.2	-	-
	1:2	131.0	126.59	103.5
	1:4	66.20	65.51	101.1
	1:8	30.94	32.76	94.46
	1:16	16.54	16.38	101.0
S5	-	277.8	-	-
	1:2	140.9	138.88	101.5
	1:4	71.29	69.44	102.7
	1:8	37.29	34.72	107.4
	1:16	19.39	17.36	111.7

		EDTA plasma		
EDTA plasma	**Dilution**	**Measured (nmol/L)**	**Expected (nmol/L)**	**Ratio (%) Measured/ Expected**
P1	-	166.1	-	-
	1:2	86.84	83.03	104.6
	1:4	26.01	22.54	115.4
P2	-	188.5	-	-
	1:2	88.03	94.25	93.40
	1:4	38.73	47.13	82.19
	1:8	26.68	23.56	113.2
P3	-	290.1	-	-
	1:2	158.3	145.1	109.1
	1:4	82.47	72.53	113.7
	1:8	33.54	36.26	92.49
	1:16	20.12	18.13	111.0
P4	-	328.0	-	-
	1:2	165.8	164.0	101.1
	1:4	78.29	82.00	95.48
	1:8	35.09	41.00	85.59
	1:16	20.27	20.50	98.88
P5	-	346.2	-	-
	1:2	185.4	173.1	107.1
	1:4	90.85	86.56	105.0
	1:8	46.53	43.28	107.5
	1:16	22.71	21.64	104.9

12.3.2 Recovery test

The recovery was performed on five serum/EDTA-plasma samples. Samples were spiked by calibrators with higher concentration. Volume of added calibrator was up to 10% of volume of samples. Samples were then assayed. Results are shown in the table below:

Serum	Endogen. conc. nmol/L	Added conc. nmol/L	Expected conc. nmol/L	Measured conc. nmol/L	Ratio (%) Measured/ Expected,
S1	66.75	46.32	113.1	114.2	101.0
	65.46	90.87	156.3	142.1	90.91
	61.89	214.8	276.7	228.3	82.53
S2	67.76	46.32	114.1	122.2	107.1
	66.46	90.87	157.3	155.9	99.07
	62.84	214.8	277.6	256.9	92.55
S3	116.7	76.21	192.9	176.3	91.40
	113.0	147.7	260.7	219.9	84.37
	109.6	214.8	324.4	262.7	81.00
S4	108.2	76.21	184.4	172.8	93.71
	104.8	147.7	252.5	230.9	91.45
	101.6	214.8	316.4	268.8	84.95
S5	93.82	46.32	140.1	144.3	103.0
	92.02	90.87	182.9	170.3	93.14
	87.00	214.8	301.8	269.1	89.16

EDTA plasma	Endogen. conc. nmol/L	Added conc. nmol/L	Expected conc. nmol/L	Measured conc. nmol/L	Ratio (%) Measured/ Expected,
P1	61.57	9.27	70.84	71.75	101.3
	61.57	18.55	80.12	78.53	98.02
	61.57	37.09	98.66	92.74	94.00
P2	75.93	9.27	85.20	80.27	94.21
	75.93	18.55	94.48	90.96	96.27
	75.93	37.09	113.0	105.4	93.24
P3	73.95	9.27	83.22	82.00	98.53
	73.95	18.55	92.50	90.32	97.64
	73.95	37.09	111.0	103.4	93.08
P4	96.75	1.65	98.40	103.7	105.3
	96.75	3.24	99.99	114.0	114.0
	96.75	4.58	101.3	114.6	113.1
P5	69.49	9.27	78.76	83.01	105.4
	69.49	18.55	88.04	85.66	97.30
	69.49	37.09	106.6	110.5	103.6

12.4 Kinetics

Incubation time may be prolonged by one hour without effect on the results.

12.5 ^{125}I Characteristics

T½(^{125}I) = 1443 h = 60.14 d

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

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
Σ	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
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Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantità	Volumen/Número	Volume/Quantité