RK-8CTACE180401

Anti-hTG [I-125] RIA KIT

(REF: RK-8CT)

Description

The Anti-hTG [^{125}I] RIA system provides a direct quantitative determination of autoantibodies to thyroglobulin in human serum in the range of about 30-3000 IU/mL using 100 µL serum samples. Each kit contains materials sufficient for 100 assay tubes permitting the construction of one standard curve and the assay of 42 unknowns in duplicate.
The test is suitable for processing on automated RIA equipment.

Introduction

The human thyroglobulin (hTG) is a high molecular weight glycoprotein (605 kDa) found in the thyroid follicular cells. It plays a central role in the uptake, incorporation, and regulated biosynthesis of thyroid hormones, T4 and T3.
Thyroid disorders are, in large part, due to autoimmun origin, and anti-thyroglobulin autoantibodies were the first factor to be discovered. Anti-hTG is found in all thyroid autoimmune diseases (Hashimoto's thyroiditis, Graves' diseases), with the highest level observed in Hashimoto's thyroiditis. Anti-hTG is also characteristic of thyroid cancer, and its determination can be used for the follow up of cancer patients.
The specificity of anti-hTG autoantibodies is complex, and samples from different patients show a variable reactivity towards epitopes present in the hTG molecule.

Principle of the method

This determination is based on the competition between monoclonal antibody coated to the surface of the test tubes, and antibodies in the sample for the binding to ^{125}I-labeled TG tracer.
Samples and calibrators are incubated with ^{125}I-TG in the anti-TG coated test tubes. After incubation the contents of the tubes are aspirated and the bound activity is measured in a gamma counter.
The concentration of anti-TG is inversely proportional to the radioactivity measured in test tubes. The concentration is read off the calibration curve generated by plotting binding values against a series of calibrators containing known amount of anti-TG.

Contents of the kit

1. 1 bottle of TRACER, ready to use.
22 mL per vial, containing < 260 kBq hTG-[^{125}I] in buffer with red dye and 0.1% NaN_3.

2. 6 vials of STANDARDS$_{(0-5)}$, ready to use. 0.75 mL per vial, containing human anti-TG antibodies in human plasma with 0.1% NaN_3.
Conc.: 0, 30, 100, 300, 1000, 3000 IU/mL.

3. 2 vials of CONTROL SERA (CI; CII), ready to use. 0.75 mL per vial, containing human plasma with 0.1% NaN_3.
The concentration of control sera are specified in the quality certificate enclosed.

4.2 boxes of COATED TUBES, ready to use. 2x50 plastic tubes, 12x75 mm, coated with monoclonal anti-hTG antibody.

Pack leaflet
Quality certificate

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (100, 200µl and 1 mL), shaker, plastic foil, adsorbent tissue, gamma counter, distilled water.
Recommended tools and equipment
repeating pipettes, dispenser with reservoir (instead of the 1-mL pipette)

Specimen collection and storage

Serum samples can be prepared according to common procedures used routinely in clinical laboratory practice. Sera 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.

Preparation of reagents, storage

Store the 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.

Assay procedure

(For a quick guide)

1. Label coated tubes in duplicate for each standard (S0-S5), control sera (CI, CII) and samples (P). Optionally, label two test tubes for total count (T).
2. Pipette **100 µl** each of STANDARDS, CONTROLS and SAMPLES into the properly labelled tubes.
3. Pipette **200 µl** of TRACER into each tube.
4. Fix the test tube rack firmly onto the shaker plate. Seal all tubes with a plastic foil. Turn on the shaker and adjust an adequate speed such that liquid is constantly rotating or shaking in each tube. (min. 600 rpm recommended).
5. Incubate tubes for 2 hours at room temperature.
6. Add 1 mL of distilled water to each tube.
7. Aspirate or 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
8. Count each tube for at least 60 seconds in a gamma counter.
9. Calculate the anti-hTG concentrations of the samples as described in calculation of results or use special software.

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

	T	S0-S5	CI,CII	P
Standard		**100**		
Control			**100**	
Samples				**100**
Tracer	**200**	**200**	**200**	**200**
Shake for 2 hours at room temperature				
Distilled water		**1000**	**1000**	**1000**
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. Data obtained should be similar to those shown in Table 2.
Calculate the average counts per minute (CPM) for each pair of assay tubes.
Calculate the percent B_0/T for zero standard (S_0) by using the following equation:

$$B_0/T \ \% = 100 * S_0 / T$$

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

$$B/B_0 \ \% = 100 * (S_{1\text{-}5} \ ; \ CI\text{-}II \ ; \ P_x) / S_0$$

Using semi-logarithmic graph paper plot B/Bo(%) for each standard versus the corresponding concentration of standards.
Figure 1 shows a typical standard curve.

Determine the anti-hTG 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.

Table 2. Typical Assay Data

Tubes	Mean cpm	B/Bo%	IU/mL
T	95 246		
S0	37 435	100	
S1	28 353	75.7	
S2	18 522	49.5	
S3	12 246	32.7	
S4	8 904	23.8	
S5	6 457	17.3	
CI	15 384		155
CII	9 920		608

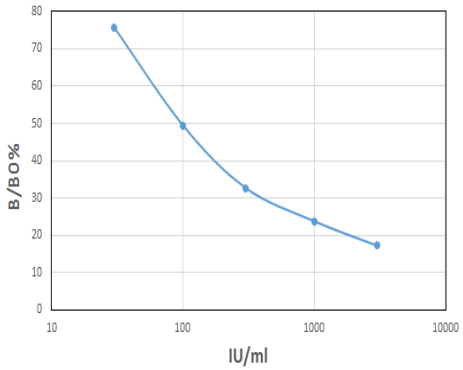

Figure 1.
A typical standard curve
(Do not use to calculate sample values)

Characterization of assay

Calibration

Standards are calibrated against the international reference standard NIBSC 65/93

Analytical sensitivity

For the analytical sensitivity 8.6 IU/mL has been obtained by assaying 20 replicates of the zero standard. The sensitivity has been determined as the concentration corresponding to the mean cpm minus its double standard deviation.

Functional sensitivity

For the functional sensitivity, 13 IU/mL was obtained, determined as the value extrapolated to 20 % of the inter-assay imprecision profile obtained from 20 independent runs of patient samples with low endogenous anti-TG concentration.

Precision and reproducibility

Seven human serum pools were assayed in 20 replicates to determine **intra-assay precision.** Values obtained are shown below.

Sample ID	Mean IU/mL	Intra-assay CV%
Pool 1	702	14.0
Pool 2	345	18.5
Pool 3	79	7.3
Pool 4	41	10.0
Pool 5	27	7.5
Pool 6	16	15.4
Pool 7	13	14.7

To determine **inter-assay precision** 7 human serum pools were measured in duplicates in 20 independent assays by 2 operators using different kit batches. Values obtained are shown below.

Sample ID	Mean IU/mL	Inter-assay CV%
Pool 1	801	16.1
Pool 2	369	12.3
Pool 3	79	7.3
Pool 4	40	7.4
Pool 5	28	8.7
Pool 6	17	11.8
Pool 7	10	24.9

Linearity – dilution test

Two patient serum samples were serially diluted with zero calibrator and anti-TG values determined. The percent recovery from the expected value of duplicate determinations was calculated. The mean recovery for two patient samples was obtained 100 and 126 %. Due to the inherent heterogeneity of the autoantibody population in human sera with regard to epitopes and affinity, some samples may not dilute linearly.

Expected values

Anti-hTG concentrations of 279 blood donors were measured. The Cut Off were calculated as a value higher than 97% of the healthy blood donor's results.

Pathological value can be assigned to *higher than 100 IU/mL* in the investigated reference population.
It is recommended that each laboratory establish its own reference intervals.
The results obtained should only be interpreted in the context of the overall clinical picture. None of in vitro diagnostic kits can be used as the one and only proof of any disease or disorder.

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.

Additional information

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

Precautions and warnings

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 antibodies to Human Immunodeficiency Virus (Anti-HIV-1/2), Hepatitis-C antibody (anti-HCV), Treponema antibody and Hepatitis-B surface Antigen (HBsAg). 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*.

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 28 mg.

Storage and shelf life

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

	Used by	LOT	Batch code
2 8°	Temperature limitation	CONTROL	Control
	Caution, consult accompanying documents	CAL	Standard
	Biological risks	CT	Coated Tube
	Consult instructions for use	TRAC	Tracer
IVD	*In vitro* diagnostic device		Manufacturer
REF	Catalogue number		Radioactive material

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