

Anti-hTG [¹²⁵I] RIA KIT

(REF: RK-8CT)

The Anti-hTG [¹²⁵I] RIA system provides a direct quantitative determination of autoantibodies to thyroglobulin in human serum in the range of about 30-3000 IU/ml using 50 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.

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 autoimmune 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 method

This determination is based on the competition between biotin labelled human polyclonal antibody and antibodies in the sample for the binding to ¹²⁵I-labelled TG tracer.

Samples and calibrators are incubated together with biotin labelled anti-TG and ¹²⁵I-TG in the streptavidin coated 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 bottle TRACER, 11 ml, ready for use. Contains less than 260 kBq of ¹²⁵I labelled hTG in buffer containing proteins, 0.1% sodium azide, red coloured.
- 1 bottle ANTISERUM, 11 ml, ready for use. Contains biotin labelled human anti-TG autoantibody in buffer containing proteins, 0.1% sodium azide, blue coloured.
- 6 vials STANDARD(S0-S5), ready for use 1x1ml (S0) and 5x0.4 ml(S1-S5), containing human anti-TG antibodies in serum with 0.1% NaN₃
Conc.: 0, 30, 100, 300, 1000, 3000 IU/ml.

- 2 vial CONTROL SERA, lyophilized

0.5 ml human serum, containing 0.1% NaN₃. The concentrations of control sera are specified in the quality certificate enclosed.

- 2 boxes COATED TUBES, ready for use. 2X50 plastic tube, coated with streptavidin.
- 1 bottle WASH BUFFER CONCENTRATE, 20 ml, containing 0.2% NaN₃. Dilute with 200 ml distilled water before use.

Quality certificate
Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (50,100µl and 2ml), shaker, plastic foil, adsorbent tissue, gamma counter

Recommended tools and equipment
repeating pipettes, dispenser with 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 48 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 of a concentration higher than 3000 IU/ml could be diluted with the zero calibrator.

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.

Add 0.5 ml distilled water to the *lyophilised control serum*, and mix gently with shaking or vortexing (foaming should be avoided). Ensure that complete dissolution is achieved, and allow the solution to equilibrate at room temperature for at least 20 minutes. For repeated use the rest of reagent can be stored at 2-8 °C until the expiry date of the kit.

Add the *wash buffer concentrate* to 200 ml distilled water. The diluted solution can be stored at 2-8 °C until expiry date of the kit. Samples having anti-TG concentrations above the measuring range can be manually diluted with standard 0. The recommended dilution is 1: 10 (20µl sample and 180µl S0).

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 serums(CI,CII) and samples(P). Optionally, label two test tubes for total count (T).
2. Pipette 50 µl each of STANDARD, CONTROL and SAMPLES into the properly labelled tubes.
3. Pipette 100 µl of ANTISERUM into each tube except T.
4. Pipette 100 µl of TRACER into each tube.
5. 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.
6. Incubate tubes for 3 hours at room temperature.
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. Add 2.0 ml of diluted WASH BUFFER to each tube.
9. Repeat Step7.
10. Count each tube for at least 60 seconds in a gamma counter.

Calculation of results

Calculate binding capacity:

$$B_0/T\% = \frac{S0 \text{ (cpm)}}{T \text{ (cpm)}} \times 100$$

Calculate percent binding for each standard, control and samples:

$$B/B_0(\%) = \frac{S1-S5/ C / P_x \text{ (cpm)}}{S0(\text{cpm})} \times 100$$

For simplicity, these values are uncorrected for non-specific binding (NSB). This is enabled by low NSB being less than 1 % of total count.

Using semi-logarithmic graph paper plot B/B₀(%) for each standard versus the corresponding concentration of standards. Determine the anti-TG concentration of the unknown samples by interpolation from the standard curve.

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

Assay Protocol, Pipetting Guide

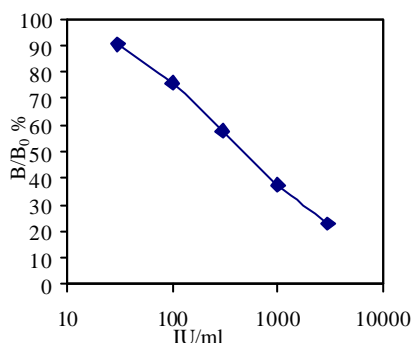
(all volumes in microlitres)

	T	S0-S5	CI,CII	P
Standard		50		
Control			50	
Samples				50
Antiserum		100	100	100
Tracer	100	100	100	100
Shake for 3 hours at room temperature				
Decant the fluid and blot on filter paper				
Wash buffer		2000	2000	2000
Decant the fluid and blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Typical assay data

Tubes	Count cpm	Mean cpm	B/Bo %	IU/ml
T	83464 84109	83787		
S0	29671 30460	30065	100	
S1	27451 26985	27218	90.5	
S2	22552 23079	22816	75.8	
S3	16588 18096	17392	57.8	
S4	11428 11110	11269	37.4	
S5	6930 6759	6845	22.7	
CI	23080 23842	23461		90.3
CII	13839 14866	14352		522

Typical standard curve



Characterization of assay

Calibration

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

Analytical sensitivity

The analytical sensitivity is 13 IU/ml, defined as the concentration corresponding of the mean cpm of zero standard minus its double standard deviation.

Functional sensitivity

This functional assay sensitivity generally represents that concentration which corresponds to the 20% between assay coefficient of variation in the respective precision profiles of the assay in lower concentration range.

The value of functional sensitivity is found to be approx. 30 IU/ml.

Precision and reproducibility

Patient samples were assayed in one run with 17 replicates, and in 8 runs with duplicates to determine the intra-assay and the inter-assay precision, respectively. Values obtained are shown below.

Intra-assay		Inter-assay	
mean (IU/ml)	CV %	Mean (IU/ml)	CV %
130	8.7	103	14.2
302	7.7	252	9.2
499	7.0	459	5.7
1090	6.9	897	9.6
		1788	9.8

Expected values

Anti-TG concentrations of 239 female and 236 male blood donors were measured.

For 10 probably not healthy patients (TSH values were beside reference interval) anti-TG values were 395±591 IU/ml.

Excluding these patients from statistical analysis, the following results were obtained:

Age(years)						
	n	Mean	SD	Min	Max	Bordeline for 95 % of the results
Female	232	34.4	11.3	18	64	-
Male	233	38.2	10.5	20	65	-
Male+female	465	36.3	11.0	18	65	-
Anti-TG (IU/ml)						
Female	232	23.2	56.5	0	433	126
Male	233	13	50.3	0	383	50
Male+female	465	18.1	53.7	0	433	101

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.

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) 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 Hepatitis B Virus, Human Immunodeficiency Virus (HIV-1), or other 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 65 mg.

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	Temperature limitation	<input type="text" value="CONTROL"/>	Control
	Caution, consult accompanying documents	<input type="text" value="CAL"/>	Standard
	Biological risks	<input type="text" value="CT"/>	Couted Tube
	Consult instructions for use	<input type="text" value="TRAC"/>	Tracer
	In vitro diagnostic device	<input type="text" value="WASHB"/>	Washing Buffer
	Manufacturer	<input type="text" value="AS"/>	Antiserum
	Catalogue number		
	Radioactive material		

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