RK-36CTACE200802

Anti-hTPO [I-125] RIA KIT

(REF: RK-36CT)

Description

The Anti-TPO [I-125] RIA system provides a direct quantitative determination of autoantibodies to thyroid peroxidase in human serum. Anti-TPO can be assayed in the range of 0-1900 IU/mL using 10 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 thyroid peroxidase (TPO), found in the thyroid follicular cells, is a high molecular weight glycoprotein (105 kDa) containing hem prosthetic group. It plays a central role in the multi-step biosynthesis of thyroid hormones, T4 and T3.

Thyroid disorders are caused in most cases due to the production of auto-antibodies against different antigens of thyroid tissues. Most important auto-antibodies are those against thyroglobulin, thyroid peroxidase and the TSH receptor.

Anti-TPO is found in all thyroid autoimmune diseases, with the highest level observed in Hashimoto's thyroiditis. Elevated concentration of anti-TPO is also characteristic of idiopathic mixoedema, and chronic atrophic thyroiditis. Anti-TPO auto-antibodies are regarded as the indicator of developing thyroiditis during pregnancy, or in patients with familiar history of different auto-immune diseases (Type-1 diabetes mellitus, Addison's disease, pernicious anemia).

Auto-antibodies to TPO are often present in patients with thyroid adenoma or thyroid carcinoma.

Principle of the method

This determination is based on the competition between human polyclonal antibody coated to the surface of the test tubes, and antibodies in the sample for the binding to ^{125}I-labelled TPO tracer.

Samples and calibrators are incubated with ^{125}I-TPO in the anti-TPO 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-TPO 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-TPO.

Contents of the kit

1. 2 bottles of TRACER, 16 ml, ready to use. Contains less than 130 kBq/ bottle of ^{125}I labelled hTPO in buffer containing proteins, 0.1 % NaN_3, red coloured.

2. 6 vials of STANDARDS (S0-S5), ready to use. 6 x 0,75 ml (S0-S5), containing human anti-TPO antibodies in human plasma with 0.1% NaN_3. Conc.: 0, 15, 50, 170, 600, 1900 IU/ml.

3. 2 vials of CONTROL SERA, ready to use. 0.75 ml human plasma, containing 0.1% NaN_3.

The concentrations of control sera are specified in the quality certificate enclosed.

4. 2 boxes of COATED TUBES, ready to use. 2x50 plastic tube, coated with human polyclonal antibody.

Pack leaflet
Quality Certificate

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (10, 300µL), 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. Samples of a concentration higher than 1900 IU/mL could be diluted with the zero calibrator.

Use of Control Sera

Good laboratory practices require that control sera be used in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and result analysis using the appropriate statistical methods is recommended.

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 **10 µL** each of STANDARDS, CONTROLS and SAMPLES into the properly labelled tubes.
3. Pipette **300 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-hTPO 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		**10**		
Control			**10**	
Samples				**10**
Tracer	**300**	**300**	**300**	**300**
Shake for 2 hours at room temperature				
Distilled water		**1000**	**1000**	**1000**
Remove the water and blot on filter paper for 2 minutes.				
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-TPO 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	82 863		
S0	42 890	100	
S1	34 946	81.5	
S2	24 827	57.9	
S3	11 200	26.1	
S4	4 404	10.3	
S5	1 823	4.3	
CI	21 880		63.7
CII	12 125		171.4

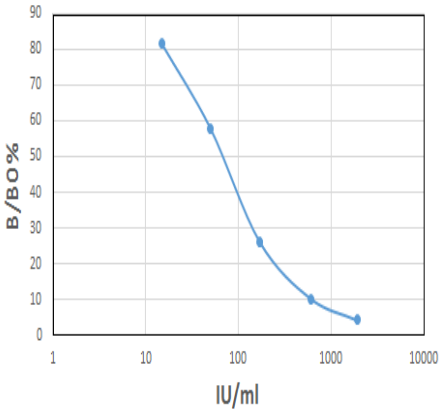

Figure 1.
A typical standard curve
(Do not use to calculate sample values)

Characterization of assay

Calibration

Standards are calibrated against the international reference standard NIBSC 66/387

Sensitivity

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined consistent with the guidelines in CLSI document EP17.

LoB = 4.60 IU/mL determined as the highest measurement result that is likely to be observed (with a stated probability [5%]) for a blank sample.
LoD = 7.26 IU/mL determined with proportions of false positives (α) less than 5 % and false negatives (ß) less than 5 %, based on 207 determinations, with 5 low level samples.
LoQ = 10.0 IU/mL as graphically determined from the precision profile curve.

Precision and reproducibility

Six 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	743	5.6
Pool 2	415	8.2
Pool 3	76	9.3
Pool 4	34	5.9
Pool 5	16	11.7
Pool 6	12	10.1

To determine **inter-assay precision** 6 human serum pools were measured in 3 replicates in 20 independent assays by 5 operators using different kit batches. Values obtained are shown below.

Sample ID	Mean IU/mL	Inter-assay CV%
Pool 1	900	9.1
Pool 2	445	6.5
Pool 3	79	6.2
Pool 4	36	8.3
Pool 5	16	10.9
Pool 6	12	15.2

Interference

No interference was observed up to the following concentration:
Bilirubin = 143 µmol/L,
Triglyceride = 27 mmol/L,
Haemoglobin = 12.2 g/L,
Biotin = 1600 ng/mL

Specificity (Cross-reaction)

No cross-reactivity with anti-hTG (2684 IU/mL) was observed.

Reference range

Anti-TPO concentrations of 279 blood donors were measured. The CutOff were calculated as a value higher than 95% of the healthy blood donor's results.

Pathological value can be assigned to *higher than 25 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.

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 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 38 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	Couted Tube
	Consult instructions for use	TRAC	Tracer
IVD	*In vitro* diagnostic device	REF	Catalogue number
	Manufacturer		Radioactive material

CE

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