

Anti-hTG [I-125] RIA KIT

(REF: RK-8CT)

Description

The Anti-hTG [¹²⁵I] RIA system provides a direct quantitative determination of autoantibodies to thyroglobulin in human serum in the range of about 0-3000 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.

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 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 ¹²⁵I-labeled TG tracer.

Samples and calibrators are incubated with ¹²⁵I-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 bottle ¹²⁵I-TRACER, ready to use.
- 22 mL per vial, containing < 260 hTG-[¹²⁵I] in buffer with red dye and 0.1% NaN₃ as preservation.
- 6 STANDARDS (0-5), ready to use.
- 0.75 mL per vial, containing human anti-TG antibodies in human plasma with 0.1% NaN₃.
- Conc.: 0, 30, 100, 300, 1000, 3000 IU/mL.

- 2 CONTROL SERA, ready to use.
- 0.75 mL per vial, containing human plasma with 0.1% NaN₃.
- The concentration of the controls are specified in the quality certificate enclosed.*
- 2 COATED TUBE, ready to use.
- 2x50 plastic tubes, 12x75 mm, coated with monoclonal anti-hTG antibody.
- Packed in plastic boxes.
- 1 Quality certificate
- 1 Pack leaflet.

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.

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 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 |
| 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 B₀/T for zero standard (S₀) 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:

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

Using semi-logarithmic graph paper plot B/B₀(%) 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 | 106 682 | - | - |
| S0 | 53 684 | 100 | 0 |
| S1 | 40 716 | 75.6 | 30 |
| S2 | 29 954 | 55.7 | 100 |
| S3 | 21 421 | 39.8 | 300 |
| S4 | 14 772 | 27.4 | 1000 |
| S5 | 11 092 | 20.6 | 3000 |
| CI | 33 344 | 62.0 | 69 |
| CII | 24 155 | 44.4 | 205 |

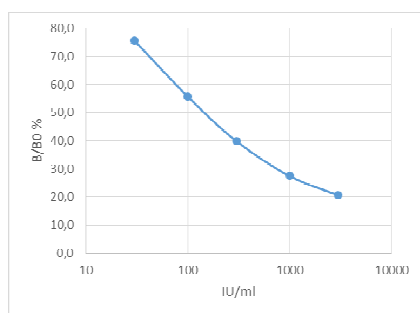


Figure 1.

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

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 = 3.50 IU/mL determined as the highest measurement result that is likely to be observed (with a stated probability [5%]) for a blank sample.

LoD = 8.29 IU/mL determined with proportions of false positives (α) less than 5 % and false negatives (β) less than 5 %, based on 207 determinations, with 5 low level samples.

LoQ = 13.0 IU/mL as graphically determined from the precision profile curve.

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 | 702.39 | 13.95 |
| Pool 2 | 345.12 | 18.52 |
| Pool 3 | 79.27 | 7.34 |
| Pool 4 | 40.82 | 9.96 |
| Pool 5 | 26.97 | 7.52 |
| Pool 6 | 15.84 | 15.45 |
| Pool 7 | 12.67 | 14.73 |

To determine **inter-assay precision** 7 human serum pools were measured in 3 replicates in 20 independent assays by 3 operators using different kit batches. Values obtained are shown below.

| Sample ID | Mean IU/mL | Inter-assay CV% |
|-----------|------------|-----------------|
| Pool 1 | 801.0 | 16.1 |
| Pool 2 | 369.1 | 12.3 |
| Pool 3 | 78.8 | 7.3 |
| Pool 4 | 40.5 | 7.4 |
| Pool 5 | 28.3 | 8.7 |
| Pool 6 | 17.4 | 11.7 |
| Pool 7 | 10.4 | 24.9 |

Reference range

Anti-hTG concentrations of 279 blood donors were measured. The Cut Off were calculated as a value higher than 95% 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.

Interference

No interference was observed up to the following concentration:

Bilirubin = 87 μ mol/L,
Triglyceride = 27 mmol/L,
Haemoglobin = 12.2 g/L,
Biotin = 1600 ng/mL

Specificity (Cross-reaction)

No cross-reactivity with anti-hTPO (1787 IU/mL) was observed.

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 | <input type="text" value="LOT"/> | Batch code |
|  | 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"/> | Coated Tube |
|  | Consult instructions for use | <input type="text" value="TRAC"/> | Tracer |
| <input type="checkbox" value="IVD"/> | In vitro diagnostic device |  | Manufacturer |
| <input type="checkbox" value="REF"/> | Catalogue number |  | Radioactive material |



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