RK-950CTA120806

[^{125}I]-T_3 UPTAKE RIA KIT

(REF: RK-950CT)

The ^{125}I-T_3 UPTAKE RIA system provides quantitative *in vitro* determination T_3 Uptake in human serum. T_3 Uptake can be assayed using 20 µl serum samples.

Principle of method

The T_3 Uptake radioimmunoassay is based on the determination of unsaturated binding capacity of TBG in human serum. Reference serum, control samples and patient sera are incubated in streptavidin coated tubes in the presence of biotinylated anti-T_3 monoclonal antibodies and ^{125}I-labeled T_3 tracer. The amount of radioactive T_3 bound to the antibody is indirectly proportional to the amount of unsaturated binding sites on TBG. After a one hour incubation the biotinylated antibodies are fixed to the test tube wall and the reaction mixture is aspirated or decanted in order to remove free iodine labeled T_3 tracer. Radioactivity bound to the reactive sites of the test tube walls is then determined in a gamma counter. The T_3 Uptake value of the patient serum samples should be calculated from the patient sample's CPM and the reference sample's CPM using a predefined percentage T_3 Uptake value of the reference serum.

Contents of the kit

1. 1 bottle TRACER. Ready to use. 11 ml per vial, containing < 260 kBq ^{125}I-T_3 in buffer with red dye and 0.1 % NaN_3.

2. 1 bottle ANTISERUM. Ready to use. 11 ml per vial, containing biotinylated anti-T3 antibodies in buffer with blue dye and 0.1 % NaN_3.

3. 1 vial lyophilised REFERENCE SERUM in human serum. To be reconstituted with distilled water, 0.5 ml/vial, containing 0.1% NaN_3. The predefined T_3 uptake value is indicated in the quality certificate.

4. 2 vials CONTROL SERUM, lyophilised. Low (CI), and high (CII). To be reconstituted with distilled water, 0.5 ml/vial, containing 0.1% NaN_3. The concentration of the control sera are specified in the quality certificate enclosed.
See *Preparation of reagents.*

5. 2 boxes COATED TUBE, Ready to use.
2x50 reactive test tubes, 12x75 mm, packed in plastic boxes.

Quality certificate
Pack leaflet

Materials, tools and equipment required

Test tube rack, precision pipettes with disposable tips (20, 100 and 2000 µl), distilled water, vortex mixer, shaker, plastic foil, adsorbent tissue, gamma counter

Recommended tools and equipment

repeating pipettes (e.g. Eppendorf or else), dispenser with 1-L 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 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

Add 50 µl distilled water to the lyophilised control sera and reference serum. 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. Store at -20°C until expiry date.
Store the rest of 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.

The assay procedure

*(For a quick guide , refer to **Table 1**.)*

1. Equilibrate reagents & samples to room temperature before use.
2. Label coated tubes in duplicate for reference serum, control sera & samples.
3. Homogenize all reagents & samples by gentle mixing to avoid foaming.
4. Pipette 20 µl of reference serum, controls & samples into the properly labeled tubes. Use rack to hold the tubes. Do not touch or scratch the inner bottom of the tubes with pipette tip.
5. Pipette 100 µl of tracer into each tube. (Set aside 2 tubes for total counts.)
6. Pipette 100 µl of antiserum into each tube.
7. Fix the test tube rack firmly onto the shaker plate. Turn on the shaker and adjust an adequate speed so that liquid is constantly rotating or shaking in each tube.
8. Incubate tubes for 1 hour, shaking at room temperature. (800 rpm recommended).
9. Add 2.0 ml of distilled water to each tube. 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.
10. Count each tube for at least 60 seconds in a gamma counter.
11. Calculate T_3 Uptake values.

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

Tubes	Total	Ref. serum	Control	Sample
Ref. serum		**20**		
Control			**20**	
Sample				**20**
Tracer	**(100)**	**100**	**100**	**100**
Antiserum		**100**	**100**	**100**
Shake for 1 hour at room temperature				
Dist.Water		**2000**	**2000**	**2000**
Decant the fluid & blot on filter paper				
Count radioactivity (60 sec/tube)				
Calculate the results				

Calculation of results

The calculation is illustrated using representative data. The assay data collected should be similar to those shown in ***Table 2.***
Calculate the average count per minute (cpm) for each pair of assay tubes.
Calculate the % uptake for each control & sample respectively by using the following equation:

$$\text{Uptake \%} = \frac{\text{Cntrl, Sample (cpm)}}{\text{Ref. serum (cpm)}} \times \text{Ref. serum \%}$$

Table 2. Typical assay data

Tubes	Count cpm	Mean cpm	T3 Uptake %
Total	81 056 79 641	80 349	-
Ref. Serum	47 412 47 303	47 358	Def. 37.0
CI	41 900 42 052	41 976	32.8
CII	62 423 62 262	62 342	48.7

Characterization of assay

Precision

The within-assay (intra-assay) precision was determined with 20 replicates within a single run using pooled human serum samples. CV values are summarized below:

Table 3.

	intra-assay	
sample No.	mean T_3 Uptake %	CV %
1	37.0	1.5
2	27.9	2.0
3	40.5	2.0
4	32.8	1.2
5	48.7	1.2

Expected Values

Expected values were obtained by testing 429 healthy blood donors. Expected euthyroid range is 29 % - 41 %. Mean value is 35,4 %.
It is recommended that each laboratory determine a reference range for euthyroids for its own patient population, since this may vary in different laboratories or regions.

Additional information

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

Note for the shaking step: The rack must hold the tubes tight during shaking in order to shake all the tubes with the same speed and strength.

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 distillation water.** For the addition of distillation water 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.

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), Hepatitis B surface Antigen (HBsAg) and Treponema Antibody.
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 23.5 mg.

LEGEND

	Use by	CONTROL	Control
LOT	Batch code	CAL	Ref. Serum
	Caution, consult accompanying documents	CT	Coated tube
	Biological risk	TRAC	Tracer
	Consult operating instructions	AS	Antiserum
IVD	In vitro diagnostic medical device		Temperature limitation Store between 2-8°C
	Manufacturer	REF	Catalogue number
	Radioactive Material		
			

Website:http://www.izotop.hu
Technical e-mail:immuno@izotop.hu
Commercial e-mail: commerce@izotop.hu

INSTITUTE OF ISOTOPES CO. LTD.
1535 Budapest. Pf.: 851.
Tel.: 36-392-2577, Fax: 36-395-9247