RiaRSR™ TRAb CT

TSH Receptor Autoantibody Coated Tube Kit – Instructions for use

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INTENDED USE

The RSR TSH receptor autoantibody (TRAb) Coated Tube (CT) kit is intended for use by professional persons only for the quantitative determination of thyrotropin receptor autoantibodies in human serum. Hyperthyroidism in Graves' disease is due to the presence of autoantibodies to the TSH receptor and measurement of these autoantibodies can be useful in disease diagnosis and management.

REFERENCES

J. Sanders et al
The Interaction of TSH Receptor Autoantibodies with ^{125}I-Labelled TSH Receptor
J Clin Endocrinol Metab 1999 **84**: 3797-3802

ASSAY PRINCIPLE

In RSR's TRAb CT kit TSH receptor autoantibodies in patient sera, calibrators and controls are allowed to interact with TSH receptor coated onto plastic tubes. After a 2 hour incubation, the samples are discarded leaving TRAb bound to the immobilised TSH receptor. Porcine TSH labelled with ^{125}I is added in a 2nd incubation step, where it interacts with TSH receptors which have not been blocked by bound TRAb. Any unbound ^{125}I-labelled TSH is then removed from the tubes by a wash step prior to counting on a gamma counter. A lower level of radioactivity bound indicates the presence of TRAb in the test sample. The measuring range is 1 – 40 IU/L (NIBSC 08/204).

STORAGE AND PREPARATION OF TEST SERUM SAMPLES

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at or below –20°C. 0.2 mL is sufficient for one assay (duplicate 100 µL determinations are recommended). Repeated freeze thawing or increases in storage temperature must be avoided. Incorrect storage of serum samples can lead to loss of TRAb activity. Do not use lipaemic or haemolysed serum samples. Do not use plasma in the assay. When required, thaw test sera at room temperature (20 – 25°C) and mix gently to ensure homogeneity. Centrifuge the serum prior to assay (preferably for 5 minutes at 10 – 15,000rpm in a microfuge) to remove any particulate matter.

SYMBOLS

Symbol	Meaning
CE	EC Declaration of Conformity
IVD	In Vitro Diagnostic Device
REF	Catalogue Number
LOT	Lot Number
	Consult Instructions
	Manufactured by
Σ	Sufficient for
	Expiry Date
8°C 2°C	Store
CONTROL -	Negative Control
CONTROL +	Positive Control

MATERIALS REQUIRED AND NOT SUPPLIED

Tubes for total counts
Suitable rack for assay tubes
Pipettes capable of dispensing 50 µL, 100 µL and 1 mL
Means of diluting wash buffer
Pure water
Orbital shaker
Gamma counter

MATERIALS SUPPLIED IN 60 and 100 TUBE KITS

MATERIAL	60 Tube	100 Tube
^{125}I-labelled TSH	1 x 6.5 mL 100kBq/bottle (at manufacture)	1 x 11 mL 180kBq/bottle (at manufacture)
TSH Receptor Coated Tubes	3 x 20 tubes	5 x 20 tubes
Start Buffer	1 x 10 mL	1 x 10 mL
Calibrators	4 x 0.7 mL	4 x 0.7 mL
Negative Control	1 x 0.7 mL	1 x 0.7 mL
Positive Control	1 x 0.7 mL	1 x 0.7 mL
Concentrated Wash Solution	1 x 50 mL	1 x 50 mL

PREPARATION OF REAGENTS SUPPLIED

Store kits and all kit components (A-G) at 2–8°C.

A	**TSH Receptor Coated Tubes** 20 tubes sealed in each foil bag. (A small amount of white residue may be present in the tubes but this does not affect assay performance.)
	After opening return any unused tubes to the original foil packet and seal. Then place foil bag in the self-seal plastic bag, with desiccant provided, and store at 2-8°C for up to 2 weeks.

B	**Start Buffer** 10 mL Coloured yellow Ready for use
C	**Negative Control** 0.7 mL Ready for use
D1-4	**Calibrators** 1, 2, 8, and 40 IU/L (units are NIBSC 08/204) 4 x 0.7 mL Ready for use
E	**Positive Control (See label for range)** 0.7 mL Ready for use
F	**^{125}I-Labelled TSH** Coloured red Ready for use
G	**Concentrated Wash Solution** 50 mL
	Dilute to 500 mL with pure water before use. Store at 2–8°C up to kit expiry.

ASSAY PROCEDURE

Allow all reagents to stand at room temperature (20–25°C) for at least 30 minutes before use. A repeating type Eppendorf pipette is recommended for steps 1, 4, 5, 6 and 8.

1.	Pipette **50 µL** of start buffer (B) into each tube (A) to be used.
2.	Pipette **100 µL** of negative control (C), calibrators (D1-4), positive control (E) and patients' sera into each tube.
3.	Cover the tubes and incubate for 2 hours at room temperature on an orbital shaker (**200** shakes per min.).
4.	Pipette **1 mL** of diluted wash solution (G) into each tube and aspirate or decant each tube to waste. If decanting, allow the tubes to drain onto a clean, dry, absorbent surface for 2 minutes.
5.	Repeat wash step 4.
6.	Pipette **100 µL** of ^{125}I-labelled TSH (F) into each tube and into two empty tubes for total counts.
7.	Cover the tubes and incubate for 1 hour at room temperature on an orbital shaker (**200** shakes per min.).
8.	Repeat wash step 4 twice (except tubes for total counts). If decanting, allow the tubes to drain as above for 5 minutes.
9.	Count each tube including total count tubes for ^{125}I for 1 minute.

RESULT ANALYSIS

Express the counts bound to each tube (B) as a percentage of the counts bound in the presence of the negative control (Bo) giving the % binding. A calibration curve can be established by plotting calibrator concentration on the x-axis (log scale) against the % binding on the y-axis (linear scale).

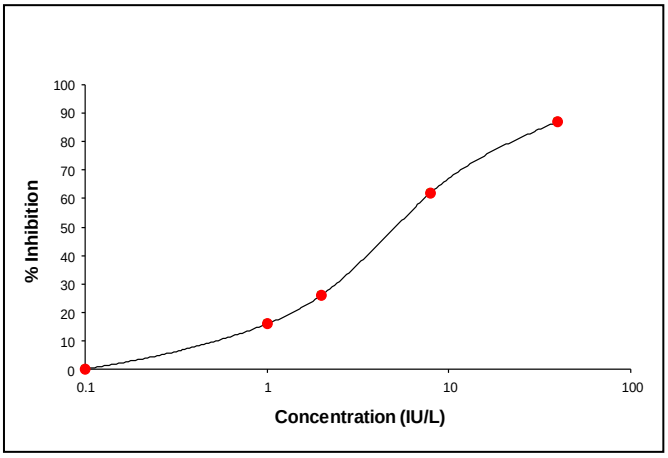
Alternatively, % Inhibition (I) can be calculated using the formula;

$$\%I = 100 \times \left(1 - \frac{B}{Bo}\right)$$

Other data reduction methods can be used. Samples with high TRAb concentrations can be diluted in kit negative control (C). For example 20 µL of sample plus 180 µL of negative control to give a 10x dilution. Other dilutions (e.g. 100x) can be prepared from a 10x dilution or otherwise as appropriate. Some sera will not dilute in a linear way and we suggest that the dilution giving a value closest to 50% inhibition is used for calculation of TRAb concentration. The negative control has a concentration of 0 IU/L, but can be assigned a value of 0.1 IU/L to facilitate computer processing of data.

TYPICAL RESULTS (example only, not for calculation of actual results)

	% B	%I	IU/L
Total Counts	83,978		
Control C	16.50	0	
D1	13.89	16	1
D2	12.24	26	2
D3	6.33	62	8
D4	2.17	87	40
Control E	9.53	42	3.9



ASSAY CUT OFF

	IU/L
Negative	≤ 1
Borderline Positive	> 1-1.5
Positive	> 1.5

This cut off has been validated at RSR. However each laboratory should establish its own normal and pathological reference ranges for TRAb levels. Also it is recommended that each laboratory include its own panel of control samples in the assay.

CLINICAL EVALUATION

Clinical Specificity

242 samples from healthy blood donors were assayed in the TRAb CT kit. 242 (100%) were identified as being negative for TSH receptor autoantibodies.

Clinical Sensitivity

50 samples from patients diagnosed with Graves' disease were assayed using the TRAb CT kit. 46 (92%) were identified as being positive for TSH receptor autoantibodies.

Lower Detection Limit

The kit negative control was assayed 20 times and the mean and standard deviation calculated. The lower detection limit at 2 standard deviations was 0.33 IU/L.

Inter Assay Precision (n = 20)

Sample	IU/L	CV (%)	%I	CV (%)
1	0.9	17	12	13
2	2.2	13	27	7.9
3	5.2	7.1	57	5.2
4	13	15	74	1.9
5	24	16	84	2.1

Intra Assay Precision (n = 25)

Sample	IU/L	CV (%)	%I	CV (%)
6	1.7	5.3	21	5.5
7	4.5	4.7	45	2.8

Clinical Accuracy

Analysis of sera from patients with autoimmune diseases other than Graves' disease indicated no interference from autoantibodies to thyroglobulin; thyroid peroxidase; glutamic acid decarboxylase; 21-hydroxylase; acetylcholine receptor; dsDNA or from rheumatoid factor.

Interference

No interference was observed when samples were spiked with the following materials; haemoglobin up to 0.5 mg/mL; bilirubin up to 0.20 mg/mL; Intralipid up to 1000 mg/dL; human LH up to 10 u/mL; hCG up to 160 u/mL; human FSH up to 15 u/mL and human TSH up to 0.3 u/L.

SAFETY CONSIDERATIONS

Follow the instructions carefully. Observe expiry dates stated on the labels and the specified shelf life for diluted reagents. Refer to Safety Data Sheet for more detailed safety information. The kit contains radioactive material ^{125}I (half-life: 60 days), emitting ionizing x-ray (28 keV) and gamma (35.5 keV) radiations. Users should make themselves aware of and observe any national and local legislation and codes of practice governing the use, storage, transportation and disposal of radioactive materials. Avoid all actions likely to lead to ingestion. Avoid contact with skin and clothing. Wear protective clothing and where appropriate personal dosimeters. Radioactive materials should only be used by authorised personnel and in designated areas. Wash hands thoroughly after handling. Monitor hands and clothing before leaving the designated area. Materials of human origin used in the preparation of the kit have been tested and found non reactive for HIV1 and 2, HCV antibodies and HbsAg but should none the less be handled as potentially infectious. Wash hands thoroughly if contamination has occurred and before leaving the laboratory. Sterilise all potentially contaminated waste, including test specimens before disposal. Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy. These materials should be handled as potentially infectious. Some components contain small quantities of sodium azide as preservative. With all kit components, avoid ingestion, inhalation, injection and contact with skin, eyes and clothing. Avoid formation of heavy metal azides in the drainage system by flushing any kit component away with copious amounts of water.

ASSAY PLAN

Allow all reagents and samples to reach room temperature (20–25 °C) before use	
Pipette:	**50 µL** start buffer
Pipette:	**100 µL** negative control, calibrators, positive control, patients' sera
Incubate	2 hours at room temperature on an orbital shaker at **200** shakes/min
Pipette:	**1 mL** wash solution
Aspirate/Decant:	Tubes
Pipette:	**1 mL** wash solution
Aspirate/Decant:	Tubes (Drain for 2 minutes)
Pipette:	**100 µL** ^{125}I-labelled TSH into each tube
Incubate:	1 hour at room temperature on an orbital shaker at **200** shakes/min
Pipette:	**1 mL** wash solution
Aspirate/Decant:	Tubes
Pipette:	**1 mL** wash solution
Aspirate/Decant:	Tubes (Drain for 5 minutes)
Count tubes in gamma counter for 1 minute	
For optimum results do not perform the assay at temperatures above 25 °C.	