RSR

RiaRSR™ TRAb

Thyrotropin Receptor Autoantibody RIA Kit - Instructions for use

CE

RSR Limited
Avenue Park Pentwyn Cardiff CF23 8HE
United Kingdom
Tel.: +44 29 2073 2076 Fax: +44 29 2073 2704
Email: info@rsrltd.com Website: www.rsrltd.com

INTENDED USE

The RSR TSH receptor autoantibody (TRAb) RIA 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

G Shrewing and B Rees Smith
"An improved radioreceptor assay for TSH receptor antibodies"
Clin. Endocrinol. 1982 **17**: 409-417
K Southgate et al
"A receptor assay for the measurement of TSH receptor antibodies in unextracted serum"
Clin. Endocrinol. 1984 **20**: 539-548
B Rees Smith et al
"Autoantibodies to the thyrotropin receptor"
Endocrine Reviews 1988 **9**: 106-121
B Rees Smith et al
"A new assay for thyrotropin receptor antibodies"
Thyroid 2004 **14**: 830-835

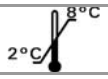
ASSAY PRINCIPLE

In RSR's TRAb RIA kit TSH receptor autoantibodies in patient sera, calibrators (optional) and controls are allowed to interact with detergent solubilised porcine 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. Bound ^{125}I-TSH is then precipitated and free ^{125}I-TSH is removed following a centrifugation step. The concentration of TRAb in a sample is read off a standard curve or expressed as an inhibition index. When using calibrators the measuring range is 10 – 405 u/L (calibrated against MRC LATS standard B).

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 -20ºC. 100 µL is sufficient for one assay (duplicate 50 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, bring test sera to room temperature (20 – 25ºC) and mix gently to ensure homogeneity. Centrifuge the serum prior to assay (preferably for 5 minutes at 10 – 15,000g 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
	Sufficient for
	Expiry Date
	Store
CONTROL -	Negative Control
CONTROL +	Positive Control

MATERIALS REQUIRED AND NOT SUPPLIED

3 mL assay tubes with suitable rack
Pipettes capable of dispensing 50 µL, 100 µL, 500 µL, 1.3 mL, 2 mL and 2.6 mL
Pure water for reconstituting freeze-dried controls (and calibrators if used)
Means of keeping reagents at 0 - 4°C
Vortex mixer
Refrigerated centrifuge capable of 1500g at 4°C
Suitable aspirating or decanting equipment
Gamma counter

MATERIALS SUPPLIED IN 50 and 100 TUBE KITS

MATERIAL	50 Tube	100 Tube
Negative Control	1 x 1.0 mL	1 x 1.0 mL
Positive Controls	2 x 0.5 mL	2 x 0.5 mL
TSH Receptor	2 x 1.3 mL	4 x 1.3 mL
Assay Buffer	1 x 10.5 mL	2 x 10.5 mL
Control Receptor	1 x 1.0 mL	1 x 1.0 mL
^{125}I-Labelled TSH	2 x 2.7 mL	4 x 2.7 mL
Precipitator	1 x 105 mL	2 x 105 mL
Calibrators (optional)	5 x 0.5 mL	5 x 0.5 mL

PREPARATION OF REAGENTS SUPPLIED

Store unopened kit and components at 2 - 8 °C.

A	**Negative Control** Ready for use
B 1-2	**Positive Control I & II** Ready for use* See controls value sheet for concentration range.
	*If the kit contains freeze-dried positive controls, reconstitute with 0.5 mL pure water. Once reconstituted, store at 2 – 8°C for the shelf life of the kit.
C	**TSH Receptor** Lyophilised
	Reconstitute each vial by addition of 1.3 mL cold (0 - 4°C) assay buffer (D) and gently mix by vortex to dissolve. **Use immediately**
D	**Assay Buffer** Ready for use Store at 0 - 4°C on day of use.
E	**Control Receptor** Ready for use
F	**^{125}I-Labelled TSH** **9 kBq/vial** Lyophilised (at manufacture)
	Reconstitute each vial by addition of 2.7 mL cold (0 - 4°C) assay buffer (D) and gently mix by vortex to dissolve. Store at 0 - 4°C and use on day of reconstitution.
G	**Precipitator** 16.5% PEG Ready for use
H 1-5	**Calibrators (optional)** 5, 15, 45, 135, 405 u/L Calibrated against MRC LATS standard B Ready for use*
	*If the kit contains freeze-dried calibrators, reconstitute with 0.5 mL pure water. Once reconstituted, store at 2 – 8°C for the shelf life of the kit.

ASSAY PROCEDURE

Ensure that ^{125}I labelled TSH, TSH receptors, assay buffer and precipitator are cold (0 - 4°C) before use. All other reagents should stand at room temperature (20 - 25°C) for at least 30 minutes before use. An Eppendorf type repeating pipette is recommended for steps 2, 4, and 6.

1.	Pipette 50 µL (in duplicate) of patient sera, negative control (A), positive controls (B1-2) and (if used) calibrators (H1-5) into labelled assay tubes. Pipette 50 µL negative control (A) into two further assay tubes (for control receptor).
2.	Reconstitute the TSH receptors (C) and immediately pipette 50 µL into each tube **except the two tubes of negative control for the control receptor**. Pipette 50 µL of control receptor (E) into these two tubes.
3.	Mix each tube on a vortex mixer; cover with a suitable cover and incubate at room temperature (20 - 25°C) for 15 minutes.
4.	Pipette 100 µL of ^{125}I-labelled TSH (F) into each tube and into two additional empty tubes for total counts.
5.	Mix each tube gently on a vortex mixer; cover the tubes with a suitable cover and incubate at room temperature (20 - 25°C) for 2 hours.
6.	Pipette 2 mL of cold (2 - 8 °C) precipitator (G) into each tube (excluding the two total count tubes) and mix thoroughly on a vortex mixer.
7.	Centrifuge each tube at 1500 g for 30 minutes at 4°C (excluding the total count tubes)
8.	After centrifugation, aspirate or decant the supernatant and count each tube (including total count tubes) for ^{125}I for 2 minutes.

RESULT ANALYSIS

Assay characteristics

Non-specific tracer binding in the assay should be less than 15% and tracer binding to receptors in the presence of negative serum should be more than 25%.

Calculation of results with calibrators

For each sample calculate the % labelled TSH bound (B_T) in relation to the total counts. Non-specific binding is the % labelled TSH bound to control receptor in the presence of negative control (B_{NSB}) and specific binding (B) = B_T - B_{NSB}.

A calibration curve can be established by plotting calibrator concentration on the x-axis (log scale) against the %B on the y-axis (linear scale). Alternatively calibrator concentration on the x-axis (log scale) can be plotted against the % inhibition of TSH binding (%I) on the y-axis (linear scale).

$\%I = [1 - (B/B_0)] \times 100$

where B_0 = specific binding of the negative control.

At RSR the calibration curve is plotted as a spline log/lin curve (smoothing factor = 0).

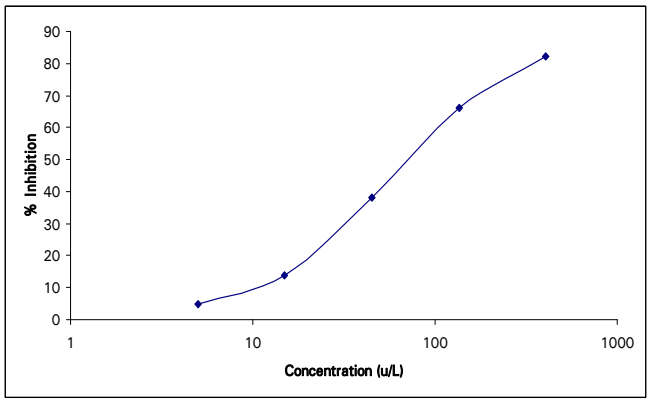
Typical results are shown below.

Calculation of results without calibrators

Assay results can be expressed in terms of inhibition of TSH binding (%I) At RSR, sera from normal subjects give values of less than 8 %I and test sera giving values greater than 10 %I are considered to contain detectable TRAb.

TYPICAL RESULTS (example only; not for use in calculation of actual results)

	% B/B0	%I	u/L
Neg Con A	1	0	
H1	0.95	5	5
H2	0.86	14	15
H3	0.62	38	45
H4	0.34	66	135
H5	0.18	82	405
Pos Con B1	0.88	12	13
Pos Con B2	0.62	38	45



ASSAY CUT OFF

Negative	< 10 u/L
Borderline Positive	≥ 10-15 u/L
Positive	≥ 15 u/L

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

307 samples from healthy blood donors were assayed in the TRAb RIA kit. 307 (100%) were identified as being negative for TSH receptor autoantibodies.

Clinical Sensitivity

108 samples from patients diagnosed with Graves' disease (treated and untreated) were assayed using the TRAb RIA kit. 67 (62%) 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 1.9 u/L.

Inter Assay Precision

Sample	u/L (n = 25)	CV (%)
1	89	8.7
2	105	10.6

Intra Assay Precision

Sample	u/L (n = 25)	CV (%)
3	40	3.6
4	24	6.0

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 or dsDNA. 40% (n = 10) of sera positive for rheumatoid factor were positive for TRAb in the RSR TRAb RIA.

Interference

No interference was observed when samples were spiked with the following materials; haemoglobin up to 5 mg/mL; bilirubin up to 20 mg/dL; 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 reconstituted reagents. Refer to Safety Data Sheet for more detailed safety information. The kit contains radioactive material. 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. Material of human origin used in the preparation of the kit has 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

Ensure tracer, TSH receptor, assay buffer and precipitator are kept at 0 – 4°C before assay. All other reagents should stand at room temperature (20 - 25°C) for at least 30 minutes before use.	
Pipette:	50 µL patient sera, negative and positive controls (and calibrators if used) including an additional 2 tubes for negative control
Pipette:	50 µL TSH receptor (**freshly reconstituted**) into all tubes except two additional tubes for negative control
Pipette:	50 µL control receptor into two additional tubes for negative control
Tubes:	Mix on vortex mixer and cover
Incubate:	15 minutes at 20 - 25°C
Pipette:	100 µL ^{125}I-labelled TSH into all tubes plus two additional empty tubes for total counts
Tubes:	Mix on vortex mixer and cover
Incubate:	2 hours at 20 - 25°C
Pipette:	2 mL precipitator into all tubes (excluding the two total count tubes)
Tubes:	Mix thoroughly on vortex mixer
Tubes:	Centrifuge at 1500 x g for 30 minutes at 4°C (excluding the two total count tubes)
Tubes:	Aspirate or decant supernatants
Count tubes using gamma counter	