RSR

ElisaRSR™ Tg

High Sensitivity ELISA for Thyroglobulin - Instructions for use

CE

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INTENDED USE

The RSR Tg ELISA is intended for use by professional persons only for the quantitative determination of serum thyroglobulin (Tg). Serum Tg measurements can be of considerable value in the management of thyroid carcinoma after initial treatment. In particular, measurable and increasing serum Tg levels are an early and reliable indicator of persistent or recurrent disease.

REFERENCES

G. Wunderlich et al
A high sensitivity enzyme-linked immunosorbent assay for serum thyroglobulin.
Thyroid 2001 **11**: 819-824

K. Zophel et al
Serum thyroglobulin measurements with a high sensitivity enzyme-linked immunosorbent assay: Is there a clinical benefit in patients with differentiated thyroid carcinoma?
Thyroid 2003 **9**: 861-865

M. Castagna et al
The use of ultrasensitive thyroglobulin assays reduces but does not abolish the need for TSH stimulation in patients with differentiated thyroid carcinoma.
J Endocrinol Invest 2011 **34**: 219-223

ASSAY PRINCIPLE

RSR's Tg ELISA is a sandwich assay in which Tg in test sera is captured by a high affinity Tg antibody coated onto ELISA plate wells. Captured Tg is then detected by addition of a second Tg antibody conjugated to horseradish peroxidase. The assay is highly sensitive with a measuring interval of 0.03 to 1000 ng/mL. The sensitivity and measuring interval of the Tg ELISA can be varied by adjusting the sample dilution and our suggestions are as follows for assays using 25 µL per well: (a) for general screening 1:100 dilution for a measuring range of 3-1000 ng/mL (b) undiluted for 0.013-10 ng/mL. Typical standard curve data for 405 nm and 450 nm is shown on page 3.

STORAGE AND PREPARATION OF SERUM SAMPLES

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at or below −20°C. 50 µL is sufficient for one assay (duplicate 25 µL determinations). Repeated freeze thawing or increases in storage temperature must be avoided. Do not use lipaemic or haemolysed serum samples. Do not use plasma in the assay. When required, thaw test sera at room temperature and mix gently to ensure homogeneity. Centrifuge serum prior to assay (preferably for 5 min at 10-15,000 rpm in a microfuge) to remove particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates. Decide on the sample dilution to be used for each test serum (see above) and make the appropriate dilutions of samples and kit controls using the sample diluent provided in the kit. Do not dilute the kit calibrators, negative control or recovery sample. Different dilutions of different test sera can be included in the same assay using the same single (undiluted) standard curve. However, if undiluted sera are to be assayed, a separate calibrator curve to which 25 µL of HAMA (human anti-mouse antibodies) blocking agent is added to each well (and to each well of undiluted test sera) must be included.

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 +	Positive Control
CONTROL -	Negative Control

MATERIALS REQUIRED AND NOT SUPPLIED

Pipettes capable of dispensing 25 µL, 100 µL and 200 µL.
Means of measuring various volumes to reconstitute or dilute reagents.
Pure water.
ELISA Plate reader suitable for 96 well formats and capable of measuring at 450 nm and 405 nm.
ELISA Plate shaker, capable of 500 shakes/min (not an orbital shaker).
ELISA Plate cover.

PREPARATION OF REAGENTS SUPPLIED

Store unopened kits and all kit components at 2–8°C.

A	**TgAb Coated Wells** 12 breakapart strips of 8 wells (96 in total) in a frame and sealed in a foil bag. Allow foil bag to stand at room temperature before opening.
	Ensure stripwells are fitted firmly into frame provided. After opening return any unused wells to the original foil bag with desiccant provided and seal with adhesive tape. Store at 2–8°C for up to 12 weeks.
B 1-8	**Calibrators** 0.03, 0.05, 0.1, 0.3, 1, 2, 3 & 10 ng/mL 8 vials Lyophilised
	Reconstitute each vial with 1.0 mL of pure water prior to use. Store at 2–8°C for up to 12 weeks.
C	**Negative Control** 1 x 1.0 mL Ready for use
D 1-2	**Positive Controls I & II (see labels for range)** 2 vials Lyophilised
	Reconstitute each vial with 1.0 mL of pure water prior to use. Store at 2–8°C for up to 12 weeks. Immediately before assay, dilute 100x with sample diluent (G).
E	**Recovery Material** 1 vial Lyophilised
	Reconstitute vial with 1.5 mL of pure water prior to use. Store at 2–8°C for up to 12 weeks.
F	**HAMA Blocking Agent** 1 x 4 mL Ready for use (only required when undiluted serum samples are assayed)
G	**Sample Diluent** 1 x 100 mL Ready for use (contains HAMA blocking agent)
H	**Conjugate Diluent** 1 x 25 mL Ready for use
I	**Conjugate** 1 vial Lyophilised
	Reconstitute with 20 mL of conjugate diluent (H). Store at 2–8°C for up to 12 weeks.
J	**Peroxidase Substrate (TMB)** 1 x 12 mL Ready for use
K	**Stop Solution (0.5M H_2SO_4)** 1 x 12 mL Ready for use
L	**Concentrated Wash Solution** 1 x 125 mL Dilute 10x with pure water before use. Store at 2–8°C for up to 12 weeks.

USE OF HAMA BLOCKING REAGENT (F)

The sample diluent (G) contains mouse IgG to block any HAMA which might be present in the test sera. In the case of assays involving undiluted sera however, 25 µL of the HAMA blocking reagent (F) supplied in the kit should be added to each well prior to addition of the undiluted test serum or kit calibrators and controls. Addition of this 25 µL of HAMA blocking agent is not necessary when diluted sera are being assayed [blocking agent present in the diluent (G)].

ASSAY PROCEDURE

Allow all reagents, to stand at room temperature (20-25°C) for at least 30 minutes before use. A repeating Eppendorf type pipette is recommended for steps 1, 5, 7 and 8.

1.	Pipette 25 µL of HAMA blocking agent (F) into each well if using undiluted samples, leaving one well empty for blank (see step 9).
2.	Pipette 25 µL of calibrators (B1-8), negative control (C), diluted positive controls (D1-2) and patient sera into respective wells in duplicate, leaving one well empty for blank (see step 9).
3.	Cover the frame and incubate at room temperature (20-25°C) for 2 hours on an ELISA plate shaker (500 shakes per min).
4.	After incubation, aspirate samples by use of a plate washing machine or discard the samples by briskly inverting the frame of stripwells over a suitable receptacle. Wash the wells three times with diluted wash solution (L) (approximately 250 µL per well) and aspirate the wash by the use of a plate washing machine or discard the wash by briskly inverting the frame of stripwells over a suitable receptacle. Tap the inverted wells gently on a clean dry absorbent surface to remove excess wash solution (not necessary when an automatic plate washer is used).
5.	Pipette 200 µL of reconstituted conjugate (I) into each well (except blank), cover the frame and incubate at room temperature (20-25°C) for 17 - 21 hours.
6.	Repeat wash step 4.

7.	Pipette 100 µL of peroxidase substrate (J) into each well (including blank) and incubate in the dark at room temperature (20-25°C) for 15 minutes without shaking.
8.	Pipette 100 µL of stop solution (K) into each well (including blank) and shake the plate for approximately 5 seconds on a plate shaker. Ensure substrate incubations are the same for each well.
9.	Within 15 minutes, read the absorbance of each well at 405nm and then 450 nm using an ELISA plate reader, blanked against the well containing 100 µL of peroxidase substrate (J) and 100 µL of stop solution (K) only.

Interference from TgAb and recovery test:
Autoantibodies to Tg, when present in test sera, can interfere in Tg assays including RSR's Tg ELISA. However, when sera are diluted 10-100 x prior to assay in the ELISA, this interference is minimal as the autoantibody concentrations are greatly reduced by dilution. In the case of assays using undiluted serum, Tg autoantibody interference is observed more frequently, as assessed by recovery test, and consequently a recovery test is particularly recommended when assays of this type are performed.

RECOVERY TEST PROCEDURE

1.	Dilute the reconstituted recovery material (E) with 1.5 mL of sample diluent (G) to give a preparation containing approximately 2.5 ng/mL Tg.
2.	To 50 µL of sample (neat or diluted) add 50 µL of recovery material (2.5 ng/mL Tg) and mix well.
3.	Assay 25 µL of test sample (neat or diluted) (x), test sample plus recovery material (y) and recovery material (z) following the assay procedure.
4.	Recovery calculation: If x = concentration of test sample, y = concentration of test sample plus recovery material and z = concentration of recovery material, then: $\%\,\text{Recovery} = \frac{y}{(z+x)/2} \times 100$
5.	Acceptable recovery range = 60 – 130%

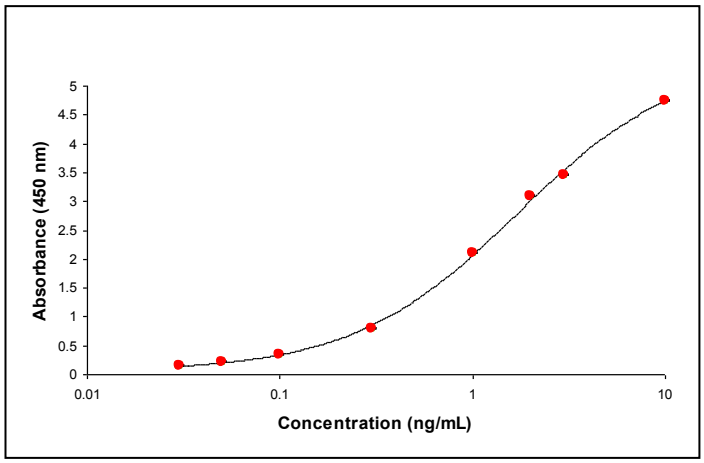
RESULT ANALYSIS
A calibration curve can be established by plotting calibrator concentration on the x-axis (log scale) against the absorbance of the calibrators on the y-axis (linear scale). Use the calibration curve to read off the concentrations of Tg in the test sera and kit positive controls, and correct the value for sample dilution. At RSR the calibration curve is plotted as a 4 parameter curve fit. Other data reduction systems can be used. The negative control can be assigned a value of 0.0001 ng/mL to assist in computer processing of assay results.

Reading the plate at 405 nm allows quantitation of high absorbances as shown in the table below. Low values should be read off the 450 nm calibration curve.

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

Calibrator ng/mL	Absorbance at 405 nm and 450 nm after overnight incubation at room temperature*	
	405 nm	450 nm
Negative Control	0.025	0.084
0.03	0.042	0.152
0.05	0.063	0.210
0.1	0.103	0.351
0.3	0.233	0.797
1	0.616	2.097
2	0.910	3.085
3	1.040	3.460
10	1.440	4.752

* Absorbance values shown have the blank values subtracted (0.053 at 405 nm; 0.049 at 450 nm).



The data in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal ranges for Tg levels.

CLINICAL EVALUATION

Normal Values
In 420 thyroid autoantibody negative healthy blood donors (36% female) Tg levels ranged from 1.5 to 590 ng/mL (mean ± SD = 33 ± 44; median = 23).

Clinical Accuracy
No interference was detected in the Tg ELISA assay from sera positive for acetylcholine receptor antibodies, TSH receptor antibodies, 21-OH antibodies, Rheumatoid factor and dsDNA antibodies.

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 0.015 ng/mL.

Functional Sensitivity

The functional sensitivity is 0.016 ng/mL (with the kit negative control assigned a value of 0.0001 ng/mL).

Inter Assay Precision

	Sample A (1:10)	Sample B (1:10)	Sample C (Undiluted)	Sample D (Undiluted)
n	20	20	20	20
[Tg] ng/mL	0.96	2.58	0.40	1.46
CV%	4.7	4.1	9.5	6.2

Intra Assay Precision

Sample	Sample E (Undiluted)	Sample F (1:10)
No of samples	21	21
[Tg] ng/mL	0.39	1.21
CV%	5.9	4.6

Calibration

The RSR Tg ELISA is calibrated against the human Tg standard CRM 457 (Community Bureau of Reference, Brussels) using the RSR Tg IRMA as reference method.

High dose hook effect

No high dose hook effect is seen up to 100 μg/mL of thyroglobulin.

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 and Intralipid up to 30 mg/mL.

SAFETY CONSIDERATIONS

Follow the instructions carefully. Observe expiry dates stated on the labels and the specified stability for reconstituted/diluted reagents. Refer to the Material Safety Data Sheet for more detailed safety information. Material of human origin used in the preparation of the kit has been tested and found non-reactive for HIV1 and 2 and 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:	For undiluted sera only, 25 μL HAMA blocking agent (F) into each well (except blank)
Pipette:	25 μL calibrators, controls and patient sera (except blank)
Incubate:	2 hours at room temperature shaking
Aspirate/Decant:	Plate
Wash:	Plate three times (and tap dry on absorbent material if manual wash)
Pipette:	200 μL conjugate (I) into each well (except blank)
Incubate:	Overnight (17–21 hours) at room temperature without shaking
Aspirate/Decant:	Plate
Wash:	Plate three times (and tap dry on absorbent material if manual wash)
Pipette:	100 μL peroxidase substrate (J) into each well (including blank)
Incubate:	15 minutes at room temperature in the dark
Pipette:	100 μL stop solution (K) into each well (including blank) and shake for 5 seconds
Read absorbance at 450 nm and 405 nm within 15 minutes of adding stop solution	