DRG® TSH Receptor Autoantibody (EIA-3369)

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Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

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This kit is not intended for in vitro diagnostic procedures.

INTENDED USE

The TSH receptor autoantibody (TRAb) ELISA kit is intended for use by professional persons only for the determination of thyrotropin receptor autoantibodies in human serum.

REFERENCES / Literature

1. J. Bolton et al. Measurement of thyroid stimulating hormone receptor autoantibodies by ELISA Clin. Chem 1999 45: 2285-2287
2. K. Kamijo. TSH receptor antibody measurement in patients with various thyrotoxicosis and Hashimoto's thyroiditis: a comparison of two two-step assays, coated plate ELISA using porcine TSH receptor and coated tube radioassay using human recombinant TSH receptor Endocrine Journal 2003 50:113-116
3. B. Rees Smith et al. A new assay for thyrotropin receptor autoantibodies. Thyroid 2004 14: 830-835

ASSAY PRINCIPLE

In TRAb ELISA, TSH receptor autoantibodies in patient sera, calibrators and controls are allowed to interact with TSH receptor coated onto ELISA plate wells. After a 2 hour incubation, the samples are discarded leaving TRAb bound to the immobilised TSH receptor. TSH Biotin is added in a 2nd incubation step, where it interacts with immobilised TSH receptors, which have not been blocked by the bound TRAb from patient sera, calibrators or controls. The amount of TSH Biotin bound to the plate is then determined in a third incubation step by addition of streptavidin peroxidase, which binds specifically to Biotin. Excess unbound streptavidin peroxidase is then discarded and the addition of tetramethylbenzidine (TMB) substrate results in formation of a blue colour.
This reaction is stopped by the addition of stop solution causing the well contents to turn from blue to yellow. The absorbance of the yellow reaction mixture at 450nm is then read using an ELISA plate reader. A lower absorbance indicates the presence of TRAb in the test sample as TRAb inhibits the binding of TSH biotin to TSH receptor coated plate wells.
The measuring range is 1 – 40 u/L (NIBSC 90/672).

STORAGE AND PREPARATION OF SERUM SAMPLES

Sera to be analyzed should be assayed soon after separation or stored, preferably in aliquots, at or below –20°C.
150 µL is sufficient for one assay (duplicate 75 µL determinations).
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 and mix gently to ensure homogeneity

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MATERIALS REQUIRED AND NOT SUPPLIED

- Pipettes capable of dispensing 50 µL, 75 µL and 100 µL
- Means of measuring out various volumes to reconstitute or dilute reagents
- Pure water
- ELISA plate reader suitable for 96 well formats and capable of measuring at 450 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 (A-K) at 2 – 8°C.

1.	**A**	**TSH Receptor Coated Wells** 12 breakapart strips of 8 wells (96 in total) in a frame and sealed in foil bag. Allow to stand at room temperature (20-25 °C) for at least 30 minutes before opening. Ensure stripwells are fitted firmly into frame provided. After opening return any unused wells to the original foil packet and seal. Then place foil bag in the self- seal plastic bag with desiccant provided. Store at 2-8°C for up to expiry of kit.
2.	**B**	**Start Buffer**, 10 mL Ready for use
3.	**C1 - 4**	**Calibrators** 1, 2, 8 and 40 U/L (units are NIBSC 90/672) 4 x 1.0 mL, Ready for use.
4.	**D1**	**Negative Control**, 1.0 mL Ready for use.
5.	**D2**	**Positive Control** (See label for range) 1.0 mL Ready for use.
6.	**E**	**TSH Biotin**, 3 x 4.5 mL Lyophilised Reconstitute each vial with 4.5 mL TSH Biotin Reconstitution Buffer (F). When more than one vial is to be used, pool the vials and mix gently before use. Store at 2 – 8°C for up to 4 weeks.
7.	**F**	**TSH Biotin Reconstitution Buffer**, 15 mL Ready for use.
8.	**G**	**Streptavidin Peroxidase (SA-POD),** 1 x 0.75 mL Concentrated. Dilute 1 in 20 with diluent for SAPOD (H). For example, 0.5 mL (G) + 9.5 mL (H). Store at 2 – 8°C for up to expiry of kit.
9.	**H**	**Diluent for SA-POD**, 15 mL Ready for use.
10.	**I**	**Peroxidase Substrate (TMB)**, 15 mL Ready for use.
11.	**J**	**Concentrated Wash Solution**, 100 mL Concentrated. Dilute to 1 liter with pure water before use. Store at 2 – 8°C up to kit expiry.
12.	**K**	**Stop Solution**, 10 mL Ready for use

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ASSAY PROCEDURE

Allow all reagents and test samples to stand at room temperature (20-25°C) for at least 30 minutes.
A repeating Eppendorf type pipette is recommended for steps 1, 5, 8, 10 and 11.
Duplicate determinations are strongly recommended for test sera, calibrators and controls.

a. Pipette **75 µL** of start buffer (B) into each well to be used, leaving the last well for a blank (see step 12).
b. Pipette **75 µL** of patient sera, calibrators (C1-4) and controls (D1 and D2) into respective wells (start with the 40 U/L calibrator and descend down the plate to the negative control and then test sera), leaving the last well blank.
c. Cover the frame and shake the wells for 2 hours at room temperature on an ELISA plate shaker (500 shakes per min.).
d. 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 once with diluted wash solution (J), and aspirate the wash by 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 (only necessary if washing plate by hand).
e. Pipette **100 µL** of reconstituted TSH biotin (E) into each well (except blank). Avoid splashing the material out of the wells during addition.
f. Cover the plate, and incubate at room temperature for 25 minutes without shaking.
g. Repeat wash step 4.
h. Pipette **100 µL** of diluted streptavidin peroxidase (G) into each well (except blank) and incubate at room temperature for 20 minutes without shaking.
i. 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 twice with diluted wash solution (J) followed by once with pure water (to remove any foam) and tap the inverted wells gently on a clean, dry, absorbent surface to remove excess wash solution (if a plate washing machine is used, the plate can be washed 3 times with diluted wash solution (J) only).
j. Pipette **100 µL** of TMB (I) into each well (including blank) and incubate in the dark at room temperature for 30 minutes without shaking.
k. Pipette **50 µL** stop solution (K) to 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.
l. Read the absorbance of each well at 450 nm using an ELISA plate reader, blanked against the well containing **100 µL** of TMB (I) and 50**µL** stop solution (K) only.

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).
The TRAb concentrations in patient sera can then be read off the calibration curve.
Other data reduction systems can be used.

Results can also be expressed as inhibition (%I) of TSH binding calculated using the formula;

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$$100 \times \left[1 - \frac{\text{test sample absorbance at 450 nm}}{\text{negative control (D1) absorbance 450 nm}} \right]$$

Samples with high TRAb concentrations can be diluted in kit negative control (D1).
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.

TYPICAL RESULTS

(example only, not for use in calculation of actual results)

Sample	A450 (minus blank)	%I	U/L
Negative Control	2.00	0	0
C1	1.70	15	1
C2	1.50	25	2
C3	0.65	68	8
C4	0.15	93	40
Positive Control	1.26	37	3.5

SAFETY CONSIDERATIONS

- This kit is intended for in vitro use by professional persons only.
- Follow the instructions carefully.
- Observe expiry dates stated on the labels and the specified stability for reconstituted reagents.
- Refer to Materials Safety Data Sheet for more detailed safety information.
- Materials of human origin used in the preparation of the kit have 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 but 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.

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ASSAY PLAN

Allow all reagents and samples to reach room temperature (20-25 °C) before use	
Pipette:	**75 µL** Start buffer each well (except blank)
Pipette:	**75 µL** Calibrators (starting with the highest concentration and descending to lowest), kit controls, patient sera (except blank)
Incubate	2 hours at room temperature on an ELISA **plate shaker at 500 shakes/min**
Aspirate/Decant:	Plate
Wash:	Plate once on automatic washer (or wash once, invert and tap dry on absorbent material for manual washing)
Pipette:	**100 µL** TSH Biotin (reconstituted) into each well
Incubate:	25 minutes at room temperature **without shaking**
Aspirate/Decant:	Plate
Wash:	Plate once as above
Pipette:	**100 µL** SAPOD (diluted 1:20) into each well (expect blank)
Incubate:	20 minutes at room temperature **without shaking**
Aspirate/Decant:	Plate
Wash:	Plate twice and rinse with pure water and dry on absorbent material ALT Plate three times on automatic washer (or wash twice, rinse once with pure water and dry on absorbent material for manual washing)
Pipette:	**100 µL** TMB into each well (including blank)
Incubate:	30 minutes at room temperature **in the dark without shaking**
Pipette:	**50 µL** stop solution into each well (including blank) and shake for 5 seconds
Read absorbance at 450 nm	
Do not perform the assay at temperatures above 25°C.	