# DRG® TSH receptor Ab (third generation) (EIA-4834)

**Revised 28 Feb. 2011 rm (Vers. 4.1)** **USA:** RUO

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

***This kit is intended for Research Use Only.***

***Not for use in diagnostic procedures.***

## INTENDED USE

The TSH receptor (TSHR) autoantibody (TRAb) ELISA is intended for the determination of TSHR autoantibodies in human serum.

## REFERENCES / Literature

B. Rees Smith et al, A new assay for thyrotropin receptor autoantibodies
Thyroid 2004 14: 830-835

K Kamijo et al, Clinical Evaluation of 3rd Generation assay for Thyrotropin Receptor Antibodies: The M22-biotin-based ELISA initiated by Smith
Endocrine Journal 2005 52: 525-529

## ASSAY PRINCIPLE

In the ELISA, TSHR autoantibodies in sera, calibrators and controls are allowed to interact with TSH receptors coated onto ELISA plate wells. After a 2 hour incubation, the samples are discarded leaving TRAb bound to the immobilised receptor. A human monoclonal autoantibody to the TSHR labelled with biotin (M22-biotin) is added in a second incubation step, where it interacts with immobilised TSH receptors which have not been blocked by bound TRAb. The amount of M22-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 addition of 3,3',5,5'-tetramethylbenzidine (TMB) resulting in the formation of a blue colour. This reaction is stopped by 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 a test sample (as TRAb inhibit the binding of M22-biotin to TSHR coated plate wells).
The measuring range is 0.4 – 30 U/L (NIBSC 90/672).

## 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.
150 µL is sufficient for one assay (duplicate 75µL determinations).

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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 or samples containing particulates.
Do not use plasma in the assay.
When required, thaw test sera at room temperature 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. Please do not omit this centrifugation step for sera that are cloudy or contain particulates.

## MATERIALS REQUIRED AND NOT SUPPLIED

- Pipettes capable of dispensing 50 µL, 75 µL, 100 µL and appropriate volumes for diluting SAPOD (F)
- Means of diluting concentrated wash (I)
- 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 kit and all kit components (A–J) at 2–8 °C

| | |
|---|---|
| **A** | TSH Receptor Coated Wells<br>**12 breakapart strips of 8 wells (96 in total) in a frame and sealed in a foil bag. Allow to stand at room temperature (20-25 °C) for at least 30 minutes before opening.** |
| | Ensure strip wells are fitted firmly into frame provided. After opening, return any unused wells to the original foil packet and seal with adhesive tape. Place foil bag in the self-seal plastic bag with desiccant provided, at 2-8°C for up to 12 weeks. |
| B | **Start Buffer**<br>10 mL<br>Coloured yellow<br>Ready for use |
| C1 - 5 | **Calibrators**<br>0.4, 1, 2.5, 10 and 30 U/L (units are NIBSC 90/672)<br>5 x 1.0 mL<br>Ready for use |
| D1 | **Negative Control**<br>1.0 mL<br>Ready for use |

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|---|---|
| D2 | **Positive Control**<br>**(See label for concentration range)**<br>1.0 mL<br>Ready for use |
| E | **M22-Biotin**<br>15 mL<br>Coloured red<br>Ready for use |
| F | **Streptavidin Peroxidase (SA-POD)**<br>1 x 0.75 mL<br>Concentrated |
| | Dilute 1 in 20 with diluent for SA-POD (G).<br>For example, 0.5 mL (F) + 9.5 mL (G).<br>Store at 2 – 8 °C after dilution for up to kit expiry date. |
| G | **Diluent for SA-POD**<br>15 mL<br>Ready for use |
| H | **Peroxidase Substrate (TMB)**<br>15 mL<br>Ready for use |
| I | **Concentrated Wash Solution**<br>100 mL<br>Concentrated |
| | Dilute to 1 litre with pure water before use.<br>Store at 2 – 8 °C up to kit expiry. |
| J | **Stop solution**<br>10 mL<br>Ready to 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 & 11.
Duplicate determinations are strongly recommended for test sera, calibrators and controls.

1. Pipette **75 µL** of start buffer (B) into each well to be used, leaving the last well for a blank (see step 12).
2. Pipette **75 µL** of calibrators (C1-5), controls (D1 and D2) and test sera into respective wells (start with the 30 U/L standard and descend down the plate to the negative control and then test sera) (except blank).
3. Cover the frame and shake the wells for 2 hours at room temperature (20 – 25 °C) on an ELISA plate shaker (500 shakes per min.).
4. Aspirate well contents by use of a plate washing machine or discard by briskly inverting the frame of stripwells over a suitable receptacle. Wash the wells once by addition of diluted wash solution (I) and aspirating 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).
5. Pipette **100 µL** of M22-biotin (E) into each well (except blank). Avoid splashing the material out of the wells during addition.
6. Cover the plate, and incubate at room temperature for 25 minutes without shaking.
7. Repeat wash step 4.
8. Pipette **100 µL** of diluted streptavidin peroxidase (F) into each well (except blank) and incubate at room temperature for 20 minutes without shaking.
9. Aspirate well contents by use of a plate washing machine or discard by briskly inverting the frame of stripwells over a suitable receptacle. Wash the wells twice with diluted wash solution (I) 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 (I) only).
10. Pipette **100 µL** of TMB (H) into each well (including blank) and incubate in the dark at room temperature for 30 minutes without shaking.
11. Pipette **50 µL** stop solution (J) into each well (including blank) and shake the plate for approximately 5 seconds on a plate shaker. Ensure substrate incubation times are the same for each well.
12. Read the absorbance of each well at 450nm using an ELISA plate reader, blanked against the well containing **100 µL** of TMB (H) and **50 µL** stop solution (J) only.

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## 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 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 M22 binding calculated using the formula;

$$100 \times \left[ 1 - \frac{\text{Test sample absorbance at 450 nm}}{\text{Negative control absorbance at 450 nm}} \right]$$

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 | Absorbance at 450nm (minus blank) | %I | U/L |
|---|---|---|---|
| Control D1 | 2.290 | | |
| C1 | 1.987 | 13 | 0.4 |
| C2 | 1.618 | 29 | 1 |
| C3 | 1.108 | 52 | 2.5 |
| C4 | 0.261 | 89 | 10 |
| C5 | 0.079 | 97 | 30 |
| Control D2 | 1.488 | 35 | 1.3 |

## ASSAY CUT OFF

| **Cut off:** | **U/L** |
|---|---|
| Negative | < 0.4 U/L |
| Positive | ≥ 0.4 U/L |

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## SAFETY CONSIDERATIONS

- 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.
- 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 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.

## ASSAY PLAN

| | |
|---|---|
| Allow all reagents and samples to reach room temperature (20-25 °C) before use | |
| Pipette: | **75 µL** Start buffer into each well (except blank) |
| Pipette: | **75 µL** calibrators (starting with the highest concentration and descending to the |
| | lowest), kit controls, 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 m for manual washing) |
| Pipette: | **100 µL** M22-biotin into each well (except blank) |
| 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 (except blank) |
| Incubate: | 20 minutes at room temperature **without shaking** |

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|---|---|
| Aspirate/Decant: | Plate |
| Wash: | 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.** |

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