RSR

FOR RESEARCH USE ONLY

ASSAY SERVICE

THYROID STIMULATING AUTOANTIBODY BIOASSAY

BioassayRSR™ TSAb

General information

Description: Bioassay for the determination of TSHR stimulating autoantibodies (TSAb) in serum.

Disease reference: Graves' disease

Advantages: Sensitive and specific bioassay

Literature:

Y Ochi et al, Thyroid 2000 **10**:653-657
Sensitive thyroid-stimulating antibody assay in whole serum containing five percent polyethylene glycol using porcine thyroid cells

B Rees Smith et al, Thyroid 2007 **17**:923-938
TSH receptor antibodies

B Rees Smith et al, Horm Metab Res 2009 41:448-455
TSH receptor – Autoantibody interactions

Sample requirement

See also **Request form for TSAb**

Assay service code: AS/TSA

Test samples: Serum from clotted blood, lipaemic or haemolysed samples are not suitable. Plasma should not be used.

Sample volume: 500µL per patient sample

Test results: 2 - 4 weeks from sample receipt.

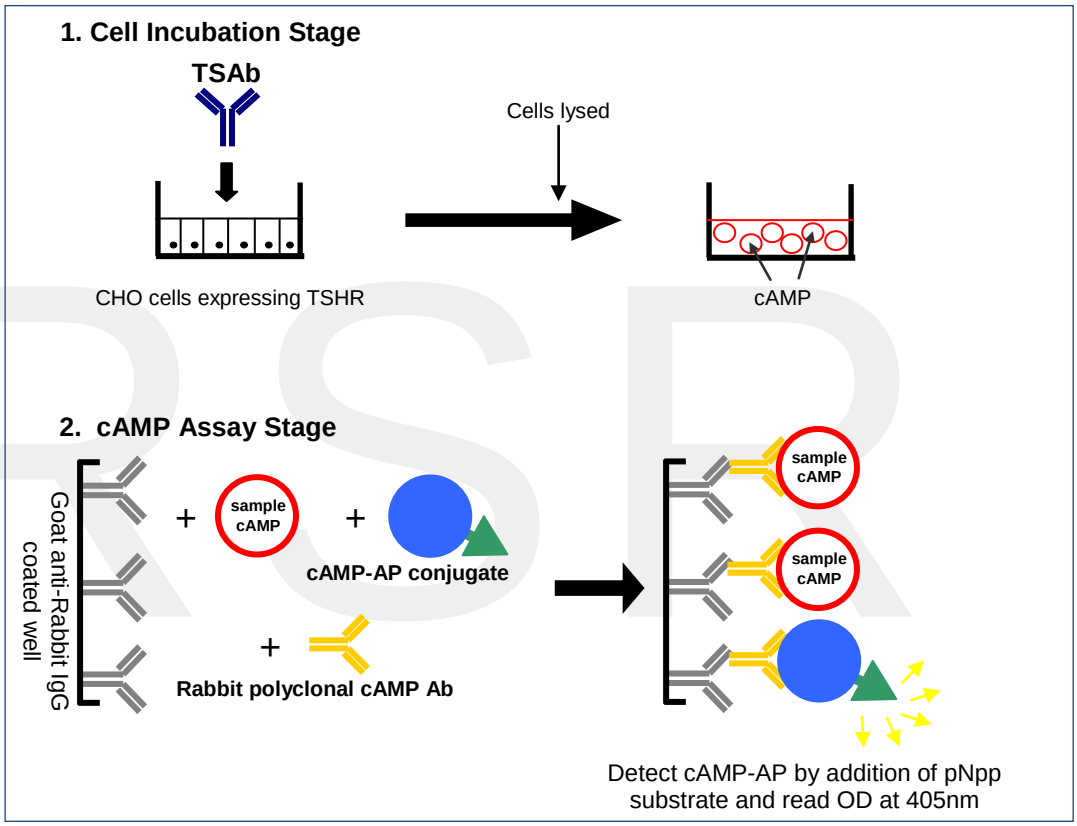
Address samples to:
Assay Service Department, FIRS Laboratories, RSR Ltd
Parc Ty Glas, Llanishen, Cardiff, CF14 5DU United Kingdom
Tel: +44 (0) 29 2076 5550
E-mail: firs-assay@rsrltd.eclipse.co.uk

RSR Limited
Diagnostics for Autoimmunity
Avenue Park Pentwyn, Cardiff, CF23 8HE United Kingdom
http://www.rsrltd.com E-mail: info@rsrltd.com
Tel: +44 (0) 29 2073 2076
Fax: +44 (0) 29 2073 2704

Technical information

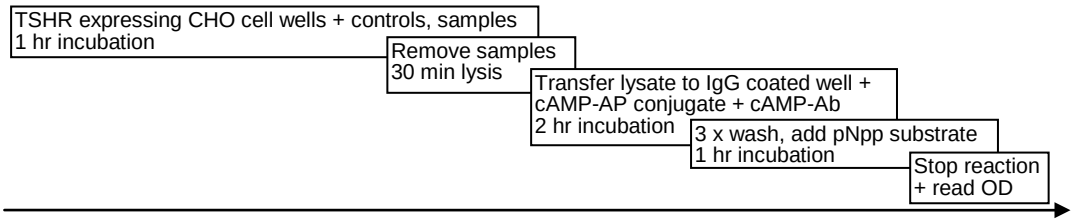
Assay method: Bioassay

Assay principle:



Sample cAMP, cAMP-AP (AP = alkaline phosphatase) conjugate and rabbit polyclonal cAMP antibody are added to goat anti-rabbit IgG coated wells where they compete for binding. Sample cAMP is detected by decreased colour development after addition of substrate.

Assay procedure:



1. Test serum samples and controls diluted 1 in 10 in buffer and added to TSHR expressing CHO cells. 1 hr incubation at 37ºC.
2. Samples removed from cell wells then cells lysed for 30 min.
3. Lysates transferred to goat anti-rabbit IgG coated wells with addition of cAMP-AP conjugate and rabbit polyclonal cAMP-Ab. 2 hr incubation.
4. Wash, add pNpp substrate. 1 hr incubation.
5. Stop reaction and read OD at 405nm.
6. Read cAMP levels off the standard curve.
7. Calculate % stimulation using the formula: -

$$\% \text{ stimulation} = \frac{\text{test serum cAMP (pmol/mL)}}{\text{pool of healthy blood donor sera cAMP (pmol/mL)}} \times 100$$

This assay service is intended for research use only. Result obtained to be used by professional persons only. The data quoted is for guidance only.

Address samples to:
Assay Service Department, FIRS Laboratories, RSR Ltd
Parc Ty Glas, Llanishen, Cardiff, CF14 5DU United Kingdom
Tel: +44 (0) 29 2076 5550
E-mail: firs-assay@rsrltd.eclipse.co.uk

RSR Limited
Diagnostics for Autoimmunity
Avenue Park Pentwyn, Cardiff, CF23 8HE United Kingdom
http://www.rsrltd.com E-mail: info@rsrltd.com
Tel: +44 (0) 29 2073 2076
Fax: +44 (0) 29 2073 2704

Assay performance

Sensitivity: 89% for Graves' disease (n = 44 treated and untreated patients positive for TRAb by ElisaRSR™ TRAb 3rd Generation and/or RiaRSR™ TRAb CT).

Specificity: 100% relative to healthy blood donors (n = 40).

Detection range: 0.2 – 50 IU/L (units: NIBSC 08/204: www.nibsc.ac.uk)

Lower detection limit: 124% stimulation (mean +3 standard deviations in assay of negative control; n = 36)

Reference cut-off: No detectable stimulating activity: <150% stimulation
Positive for stimulating activity: ≥150% stimulation

Cross reactivity: Using 150% stimulation cut-off, 0/13 Addison's disease patients, 0/20 rheumatoid arthritis patients and 0/19 type 1 diabetes mellitus patients were positive for TSAb activity

Interference: Serum TSH levels >10 mU/L (normal range approx. 0.4 – 4 mU/L) result in stimulation of cAMP production.
Serum hCG levels >90,000 mU/mL result in stimulation of cAMP production (normal levels for males and non pregnant females 0–5 mU/mL, in pregnant females levels can reach >200,000 mU/mL).
Serum LH concentrations up to 6,000 mU/mL (normal range approx. 5 -25 mU/mL) and serum FSH concentrations up to 10,000 mU/mL (normal range approx. 1.5 – 135 mU/mL) do not cause stimulation of cAMP production.
Bilirubin (20 mg/dL), haemoglobin (500 mg/dL) and lipid (3,000 mg/dL) do not interfere with the BioassayRSR™TSAb assay.
Plasma samples are not suitable for use in the BioassayRSR™TSAb assay.

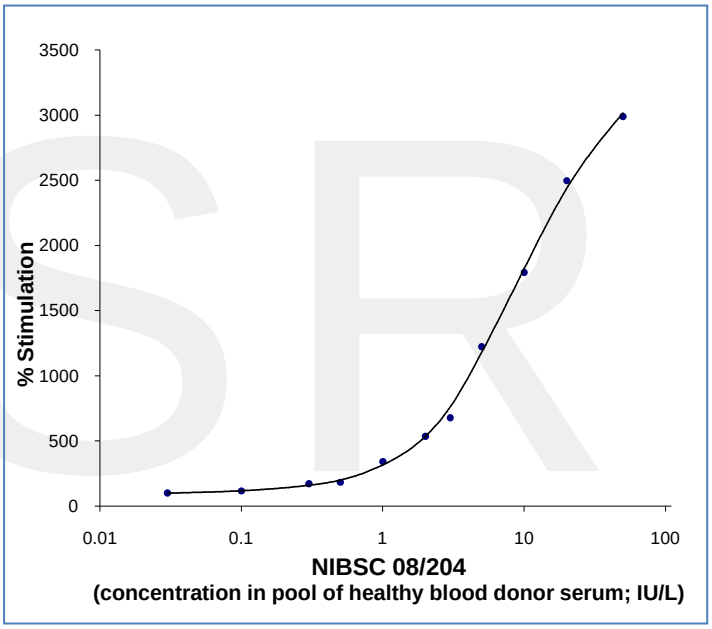
Inter assay precision:

Sample (n=20)	% stimulation	CV (%)
A	326	15.5
B	871	17.7
C	1185	17.6

Intra assay precision:

Sample (n=25)	% stimulation	CV (%)
1	330	11.7
2	523	11.8
3	920	17.1

NIBSC 08/204 curve: Dilution profile of the international standard for Thyroid Stimulating Antibody NIBSC 08/204 shows a wide dose-response range, similar to current TRAb assays based on inhibition of M22™ binding to the TSH receptor.
0.2 IU/L gives approximately 150%.

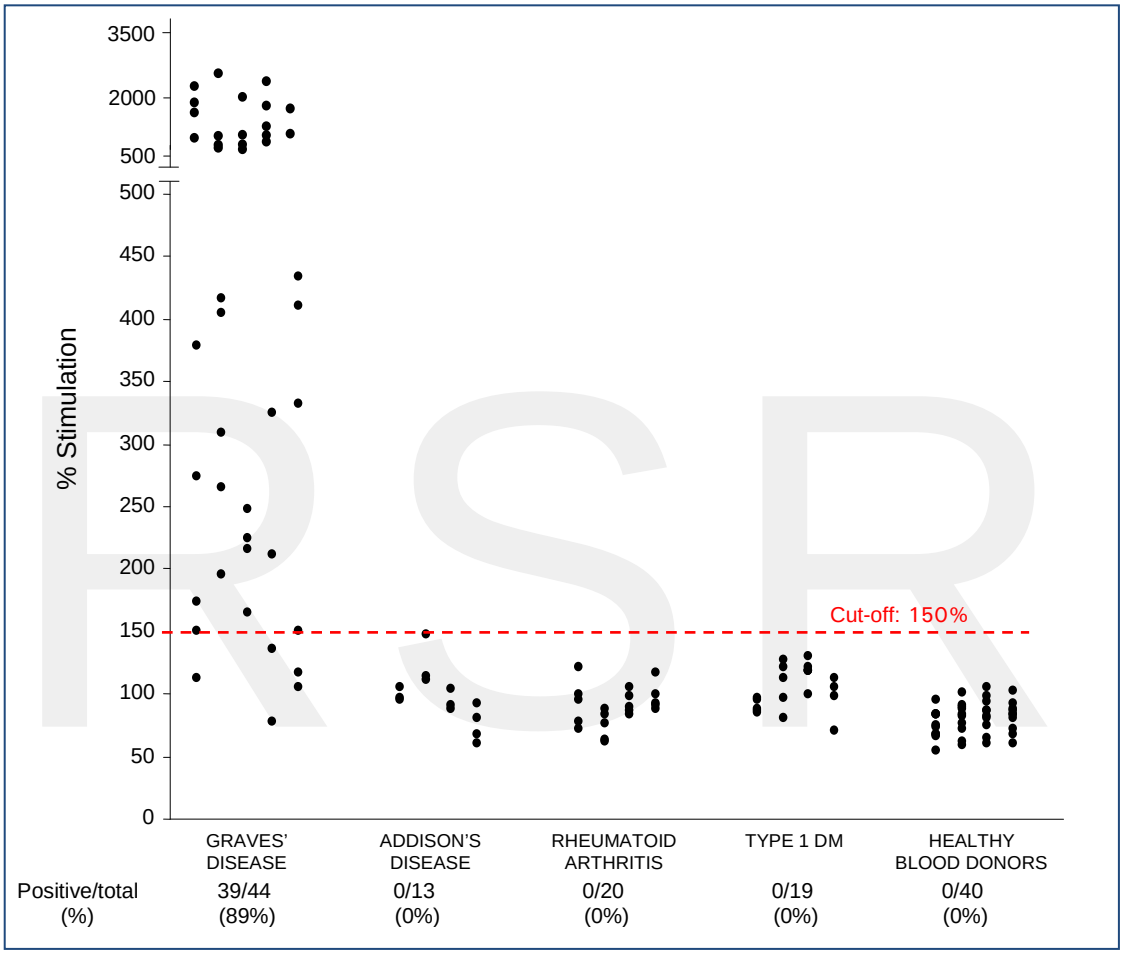


This assay service is intended for research use only. Result obtained to be used by professional persons only. The data quoted is for guidance only.

Address samples to:
Assay Service Department, FIRS Laboratories, RSR Ltd
Parc Ty Glas, Llanishen, Cardiff, CF14 5DU United Kingdom
Tel: +44 (0) 29 2076 5550
E-mail: firs-assay@rsrltd.eclipse.co.uk

RSR Limited
Diagnostics for Autoimmunity
Avenue Park Pentwyn, Cardiff, CF23 8HE United Kingdom
http://www.rsrltd.com E-mail: info@rsrltd.com
Tel: +44 (0) 29 2073 2076
Fax: +44 (0) 29 2073 2704

Measurements in different groups:



Samples from Graves' disease patients (n =44), healthy blood donors (n = 40), Addison's disease patients (n = 13), rheumatoid arthritis patients (n = 20) and type 1 diabetes mellitus patients (n = 19) were tested for TSAb using BioassayRSR™ TSAb.

RESULTS:

39/44 (89%) patients positive for TRAb tested by ElisaRSR™ TRAb 3rd Generation and/or RiaRSR™ TRAb CT were positive for TSAb.

All 40 (100%) healthy controls were identified as being negative for TSAb.

None of the Addison's disease patients (n = 13, positive for 21-OH Ab by RiaRSR™ 21-OH Ab), rheumatoid arthritis patients (n = 20, positive for rheumatoid factor) or the type 1 diabetes mellitus patients (n = 19, positive for GADAb by ElisaRSR™ GADAb) were positive for TSAb.

Other information

Significance: Thyroid stimulating autoantibodies are the cause of hyperthyroidism in Graves' disease.

Address samples to:
Assay Service Department, FIRS Laboratories, RSR Ltd
Parc Ty Glas, Llanishen, Cardiff, CF14 5DU United Kingdom
Tel: +44 (0) 29 2076 5550
E-mail: firs-assay@rsrltd.eclipse.co.uk

RSR Limited
Diagnostics for Autoimmunity
Avenue Park Pentwyn, Cardiff, CF23 8HE United Kingdom
http://www.rsrltd.com E-mail: info@rsrltd.com
Tel: +44 (0) 29 2073 2076
Fax: +44 (0) 29 2073 2704