RSR

FOR RESEARCH USE ONLY

ASSAY SERVICE

BLOCKING TYPE TSH RECEPTOR AUTOANTIBODY BIOASSAY

BioassayRSR™ TSBAb

General information

Description: Bioassay for the determination of blocking type TSH receptor (TSHR) autoantibodies (TSBAb) in serum.

Disease reference: Hypothyroidism, transient, Hashimoto's disease, myxoedema

Advantages: Sensitive and specific bioassay

Literature:

Y Ochi et al, Horm Metab Res 1999 51:142-149
Clinical usefulness of TSAb assay with high polyethylene glycol concentrations

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

Y Ochi et al, Horm Metab Res 2001 **33**:115-1120
Sensitive assay to detect thyroid stimulating antibody (TSAb) in the presence of thyroid stimulation blocking antibody (TSBAb) in serum

N Takasu et al, Horm Metab Res 2001 **33**:232-237
TSBAb (TSH -stimulation blocking antibody) and TSAb (thyroid stimulating antibody) in TSBAb –positive patients with hypothyroidism and Graves' patients with hyperthyroidism

M Evans et al, Clin Endocrinol 2010 **73**: 404-412
Monoclonal autoantibodies to the TSH receptor one with stimulating activity and one with blocking activity obtained from the same blood sample

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

P Sanders et al, J Molecular Endocrinol 2011 **46**:81-99
Crystal structure of the TSH receptor (TSHR) bound to a blocking-type TSHR autoantibody

N Takasu and M Matsushita, J Thyroid Res vol 2012, Article ID 182176, 11 pages, 2012. doi:10.1155/2012/182176
Changes of TSH–stimulation blocking antibody (TSBAb) and thyroid stimulating antibody (TSAb) over 10 years in 34 TSBAb–positive patients with hypothyroidism and 98 TSAb-positive Graves' patients with hyperthyroidism: Reevaluation of TSBAb and TSAb in TSH–receptor–antibody (TRAb)–positive patients

Sample requirement

See also **Request form for TSBAb**

Assay service code: AS/TSB

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

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

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Avenue Park Pentwyn, Cardiff, CF23 8HE United Kingdom
http://www.rsrltd.com E-mail: info@rsrltd.com
Tel: +44 (0) 29 2073 2076
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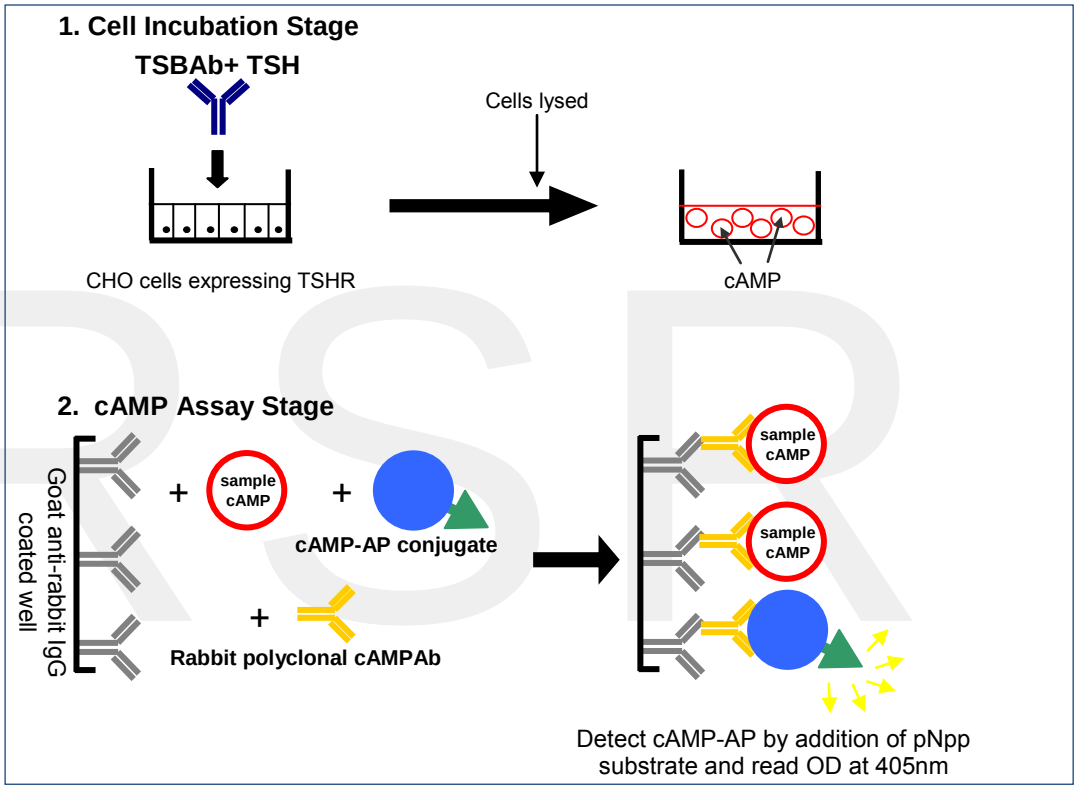
Technical information

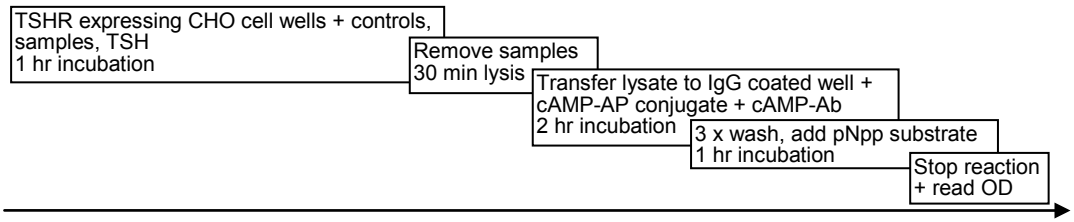
Assay method: Bioassay

Assay principle: In the assay, the inhibiting effect of TSBAb in test sample on porcine TSH induced stimulation of cAMP production by Chinese hamster ovary (CHO) cells expressing human TSHR is measured.



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 5 in buffer and added with TSH (test serum final dilution of 1 in 10) 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 % inhibition using the formula: -

$$\% \text{ inhibition} = 1 - \left(\frac{\text{cAMP level in the presence of TSH and test serum (pmol/mL)}}{\text{cAMP level in the presence of TSH and HBD}^{a} \text{ control serum (pmol/mL)}} \right) \times 100$$

[a] HBD: healthy blood donor

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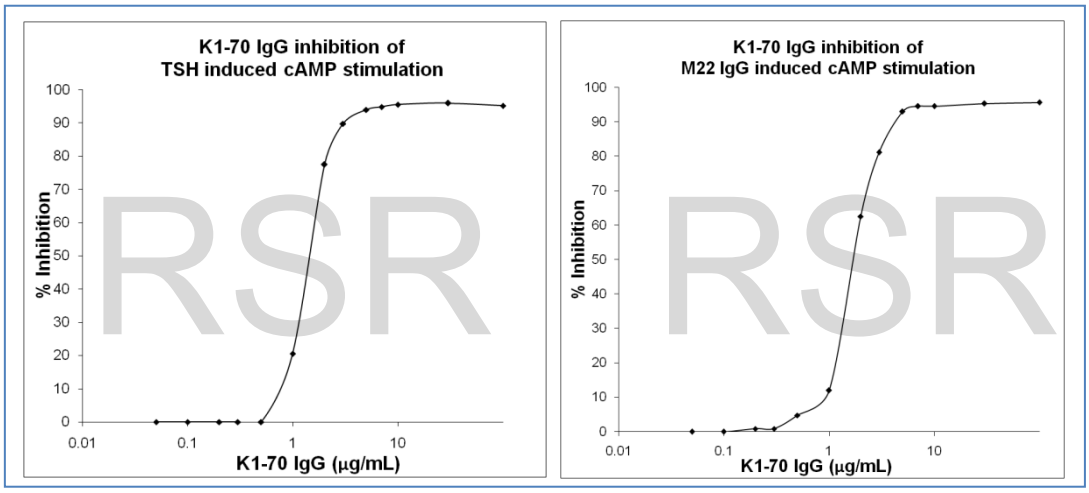
Assay performance

Detection range: Approx.1.2–10µg/mL of human monoclonal TSBAb K1-70™ IgG

Lower detection limit: 21% inhibition (mean +3 standard deviations in assay of pool of HBD sera in the presence of TSH; n = 25)

Reference cut-off: No detectable blocking activity: < 30% inhibition
Positive for blocking activity: ≥ 30% inhibition

Dilution curve:



TSH and M22™ IgG (a human thyroid stimulating monoclonal autoantibody) are potent stimulators of cAMP production in CHO cells expressing the TSHR. K1-70™ IgG inhibits both TSH and M22™ IgG induced stimulation of cAMP production in a dose-dependent manner, characterised by a steep gradient to the curve. Inhibiting effects of K1-70™ IgG on TSH and M22™ IgG induced stimulation of cAMP production are similar.

Other information

Significance: Blocking type TSHR autoantibodies can cause hypothyroidism. When present in pregnancy, they can be responsible for neonatal hypothyroidism. They can also ameliorate hyperthyroidism in patients with Graves' disease.

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