CD Creative Diagnostics®

TSHR Antibodies for Graves' Disease Assay Development

Overview

Anti-thyroid stimulating hormone receptor (TSHR) antibodies are pathophysiologic and clinical indicators of autoimmune thyroid disease. TSHR antibodies (TRAbs) can be divided into two categories. TSHR antibodies that stimulate the thyroid (TSAb) cause Graves' hyperthyroidism, while TSHR antibodies that block thyrotropin action (TBAb) are responsible for hypothyroidism. TRAbs have important clinical significance in the diagnosis, monitoring and evaluation of Graves' disease (GD).

TSHR in The Pathogenesis of GD

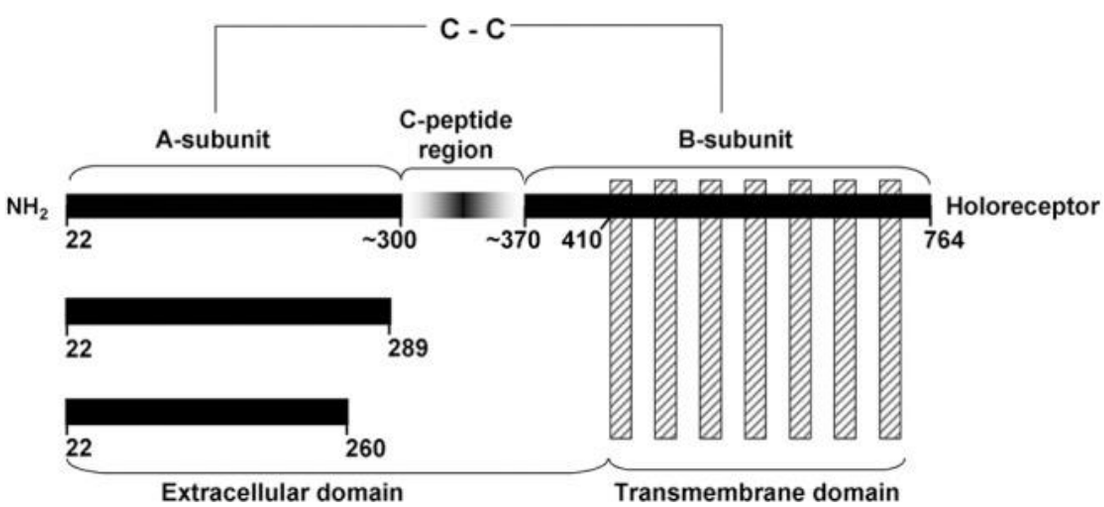


Fig. 1 Schematic representation of TSHR components.
(McLachlan SM, Rapoport B. *Thyroid.* 2013)

TSHR is a common target autoantigen of GD, and it is a 3-domain G protein-coupled receptor (GPCR). The natural ligand for TSHR under normal conditions is TSH. However, in GD, the misrecognition of TSHR results in the generation of thyroid-stimulating immunoglobulins (TSIs) that target the receptor, which is densely displayed on thyroid epithelial cells, resulting in pathologically high levels of thyroid hormones.

Autoantibodies in GD

In GD, IgG directed against TSHR are produced as a result of the autoimmune reactivity underlying the disease. The hyperthyroidism of GD is caused by TRAbs with stimulating (agonist) activity, while the rare blocking (antagonist) TRAbs are responsible for hypothyroidism in some patients. The isolation of human TSHR monoclonal antibodies (MAbs) with either stimulating (clone M22 and clone K1-18) or blocking (5C9 and K1-70) activities has been a major advance in the study of the TSHR.

• Thyroid stimulating monoclonal autoantibodies

Clone M22 was isolated from a hyperthyroid patient with GD and has potent thyroid-stimulating activity. Clone K1-18 is a hybridoma prepared from the peripheral blood lymphocytes of a patient with hypothyroidism and high levels of TSHR autoantibodies. This donor has a history of hyperthyroidism followed by hypothyroidism. Both clone M22 and clone K1-18 have high a binding affinity for the TSHR. In addition, both monoclonal antibodies are potent stimulators of cyclic AMP production at nanogram per ml concentrations, with clone M22 IgG being more effective than clone K1-18 IgG, while both antibodies are full agonists of the TSHR, providing the same maximal stimulation of cyclic AMP production as TSH. Clone M22 has great potential as an alternative to recombinant human TSH in the management of patients with thyroid cancer.

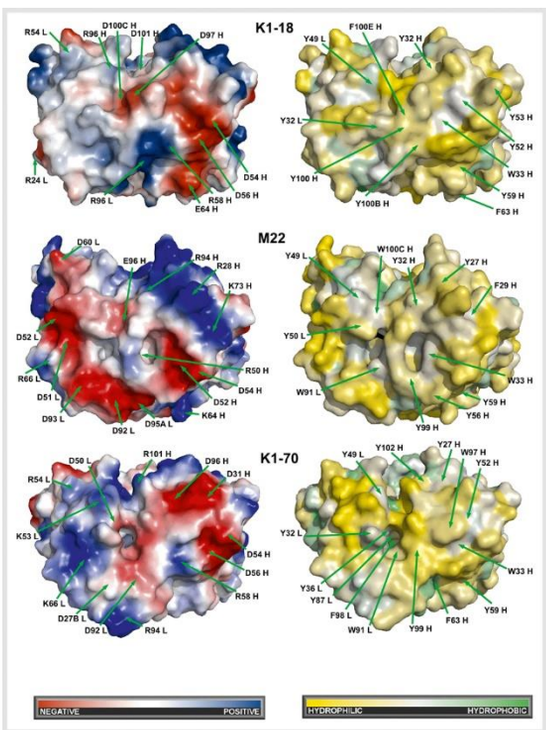


Fig. 2 The antigen binding surfaces of thyroid stimulating monoclonal autoantibodies (**K1-18** and **M22**) and TSHR blocking monoclonal autoantibody (K1-70).
(Furmaniak J.; *et al. Horm Metab Res*. 2015)

CD Creative Diagnostics®
Tel: 1-631-624-4882 (USA) / 44-161-818-6441 (Europe) Fax: 1-631-938-8221
www.creative-diagnostics.com
info@creative-diagnostics.com

- **Blocking monoclonal autoantibodies**

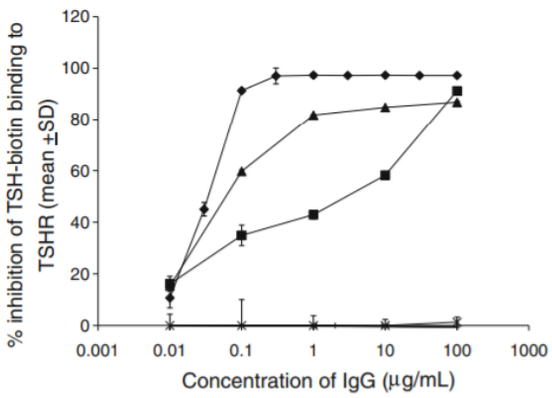


Fig 3. Inhibition of TSH-biotin binding to TSHR coated ELISA plate wells by different concentrations of **K1-70** IgG (filled diamond), **MAb-B2** IgG (filled triangle), **5C9** IgG (filled square) and negative control IgG (times symbol)
(Furmaniak J.; *et al. Auto Immun Highlights.* 2012)

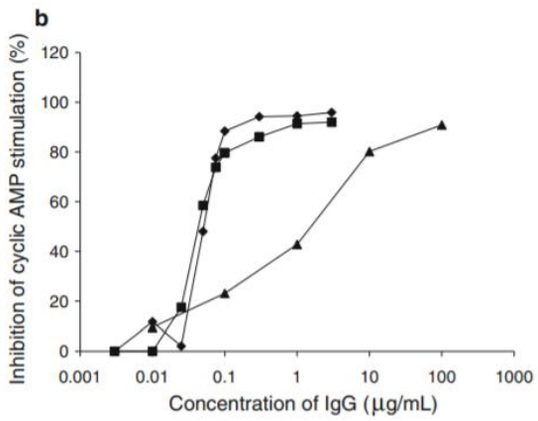


Fig 4. Inhibition of porcine TSH mediated stimulation of cyclic AMP production by TSHR monoclonal antibodies with antagonist activity. **MAb-B2** IgG (filled triangle), **K1-70** IgG (filled diamond), **5C9** IgG (filled square)
(Furmaniak J.; *et al. Auto Immun Highlights.* 2012)

Clone K1-70 was isolated from a patient with autoimmune hypothyroidism and has strong antagonist (blocking) activity. Clone 5C9 was isolated from the peripheral blood lymphocytes of a patient with postpartum hypothyroidism and high levels of TSHR autoantibodies. Clone K1-70 is the most effective inhibitor of TSH binding to the receptor, with as little as 0.1 µg/mL providing 97 % inhibition, while 0.1 µg/mL of clone 5C9 provides 35 % inhibition. Both clone K1-70 and clone 5C9 are potent inhibitors of TSH stimulation of cyclic AMP production in CHO-TSHR cells and are active at nanogram/mL concentrations.

Other Developed Antibodies

Much has been learned from the detailed characterization of the agonistic antibodies generated against TSHR, as well as those that inhibit ligand binding and thus block the actions of TSIs and TSH. Some antibodies have been developed as inverse agonists, inhibiting the constitutive activities of TSHR. For example, monoclonal antibodies that bind to several regions of the receptor, including those containing amino acids 32-41, 36-42, 246-260, 277-296, and 381-385, can block TSH binding. In addition, several monoclonal antibodies (e.g., clone B2 and clone CS-17) are effective in blocking TSH binding and TSHR activation.

TSHR Antibodies for Graves' Disease Assay Development

Applications of TSHR Antibodies

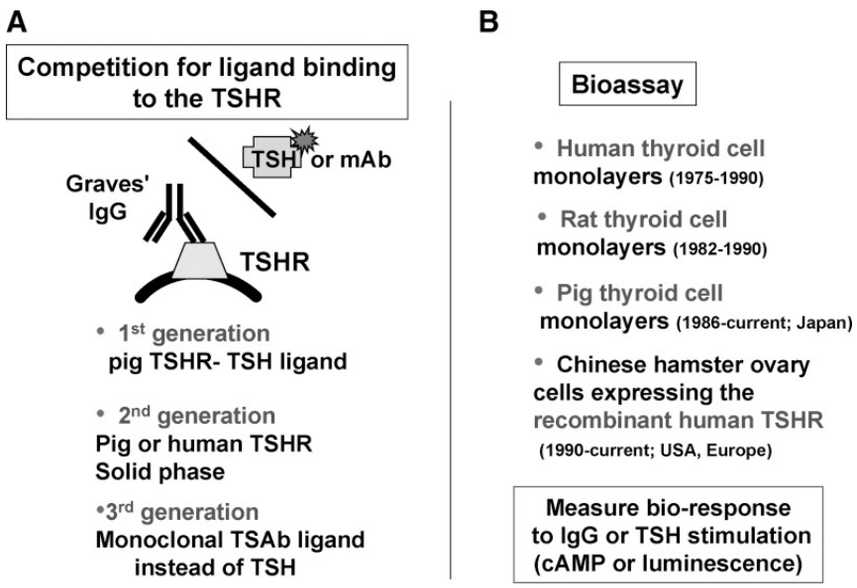


Fig. 5 Two types of thyrotropin receptor (TSHR) antibody assays.

(McLachlan SM, Rapoport B. *Thyroid.* 2013)

TSHR monoclonal antibodies are useful reagents in improved methods for detecting TRAbs in samples and as reference tools for measuring TRAb concentration and bioactivity. Beginning ~50 years ago, each type of TRAb assay has undergone extensive modifications. The 1st generation TRAb assay was based on the ability of TRAbs in test sera to inhibit the binding of radiolabelled TSH to detergent-solubilised TSHR preparations, with the labelled TSH-TSHR complexes precipitated with polyethylene glycol. In 2nd generation assays, TSHR preparations were used in solid phase rather than in solution, along with non-radioactive TSH ligand tagged with a reagent to provide a fluorescent or color signal. TSHR autoantibody competition with a tagged human monoclonal TSHR autoantibody represents a 3rd generation assay. In addition, TSHR monoclonal antibodies have potential applications in the detection of TSHR in tissue sections by immunohistochemistry. Biotinylated clone M22 or clone K1-70 showed strongly positive staining at the basal and lateral surfaces of thyroid epithelial cells, consistent with the presence of TSHR at these sites.

Product Performance

For the TSHR detection, we offer several research-grade humanized antibodies and mouse monoclonal antibodies. These clones are useful reagents in improved methods for the detection of TSHR and as reference preparations for the measurement of antibody bioactivity.

CD Creative Diagnostics®

Tel: 1-631-624-4882 (USA) / 44-161-818-6441 (Europe) Fax: 1-631-938-8221

www.creative-diagnostics.com

info@creative-diagnostics.com

TSHR Antibodies for Graves' Disease Assay Development

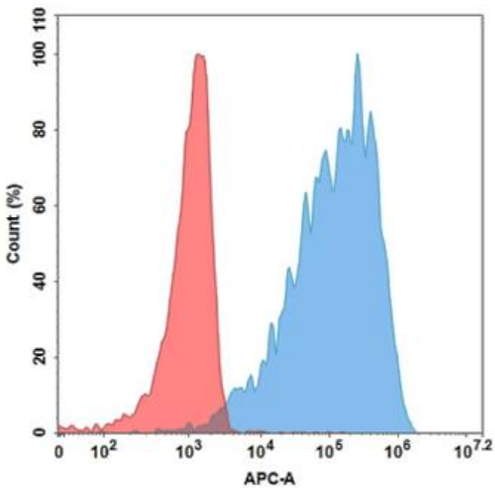


Fig. 6 Flow cytometry analysis with Anti-TSHR（M22）mAb (Cat. No **DMABB-JX42**) on Expi293 cells transfected with Human TSHR (Blue) or Expi293 transfected with irrelevant protein (Red).

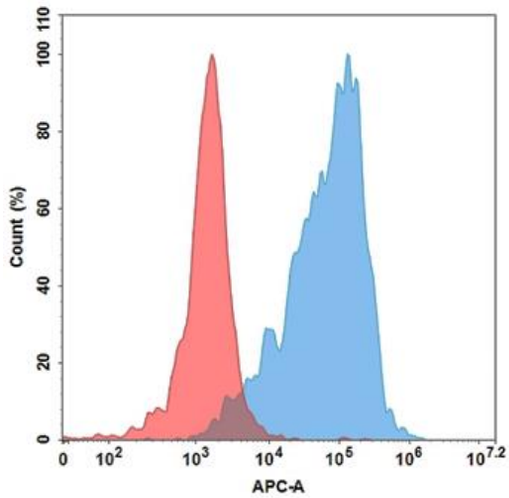


Fig. 7 Flow cytometry analysis with Anti-TSHR（K1-70）mAb (Cat. No **DMABB-JX44**) on Expi293 cells transfected with Human TSHR (Blue) or Expi293 transfected with irrelevant protein (Red).

Products List

Cat. No	Product Name	Host	Application
DMABB-JX42	Human Anti-Human TSHR monoclonal antibody, clone M22	Human	FC
DMABB-JX43	Mouse Anti-Human TSHR monoclonal antibody, clone K1-70	Mouse	BL, ELISA, FC
DMABB-JX44	Human Anti-Human TSHR monoclonal antibody, clone K1-70	Human	FC
DMABB-JX45	Mouse Anti-Human TSHR monoclonal antibody, clone 5C9	Mouse	ELISA, Neut
DMABB-JX46	Mouse Anti-Human TSHR monoclonal antibody, clone K1-18	Mouse	ELISA
DMABB-JX47	Mouse Anti-Human TSHR monoclonal antibody, clone 3BD10	Mouse	ELISA, FC, IP, WB
DMABB-JX48	Mouse Anti-Human TSHR monoclonal antibody, clone CS-17	Mouse	ELISA, FC
CABT-L6229	Human Anti-human TSHR monoclonal antibody, clone 1HA	Human	ELISA
CABT-L6230	Human Anti-human TSHR monoclonal antibody, clone 2HB	Human	ELISA

Reference

1. Smith TJ. TSHR as a therapeutic target in Graves' disease. *Expert Opin Ther Targets.* 2017 Apr;21(4):427-432.

2. Cui X.; *et al.* A review of TSHR- and IGF-1R-related pathogenesis and treatment of Graves' orbitopathy. *Front Immunol.* 2023 Jan 19;14:1062045.

CD Creative Diagnostics®

Tel: 1-631-624-4882 (USA) / 44-161-818-6441 (Europe) Fax: 1-631-938-8221

www.creative-diagnostics.com

info@creative-diagnostics.com

3. Furmaniak J.; *et al.* Mechanisms of Action of TSHR Autoantibodies. *Horm Metab Res.* 2015 Sep;47(10):735-52.

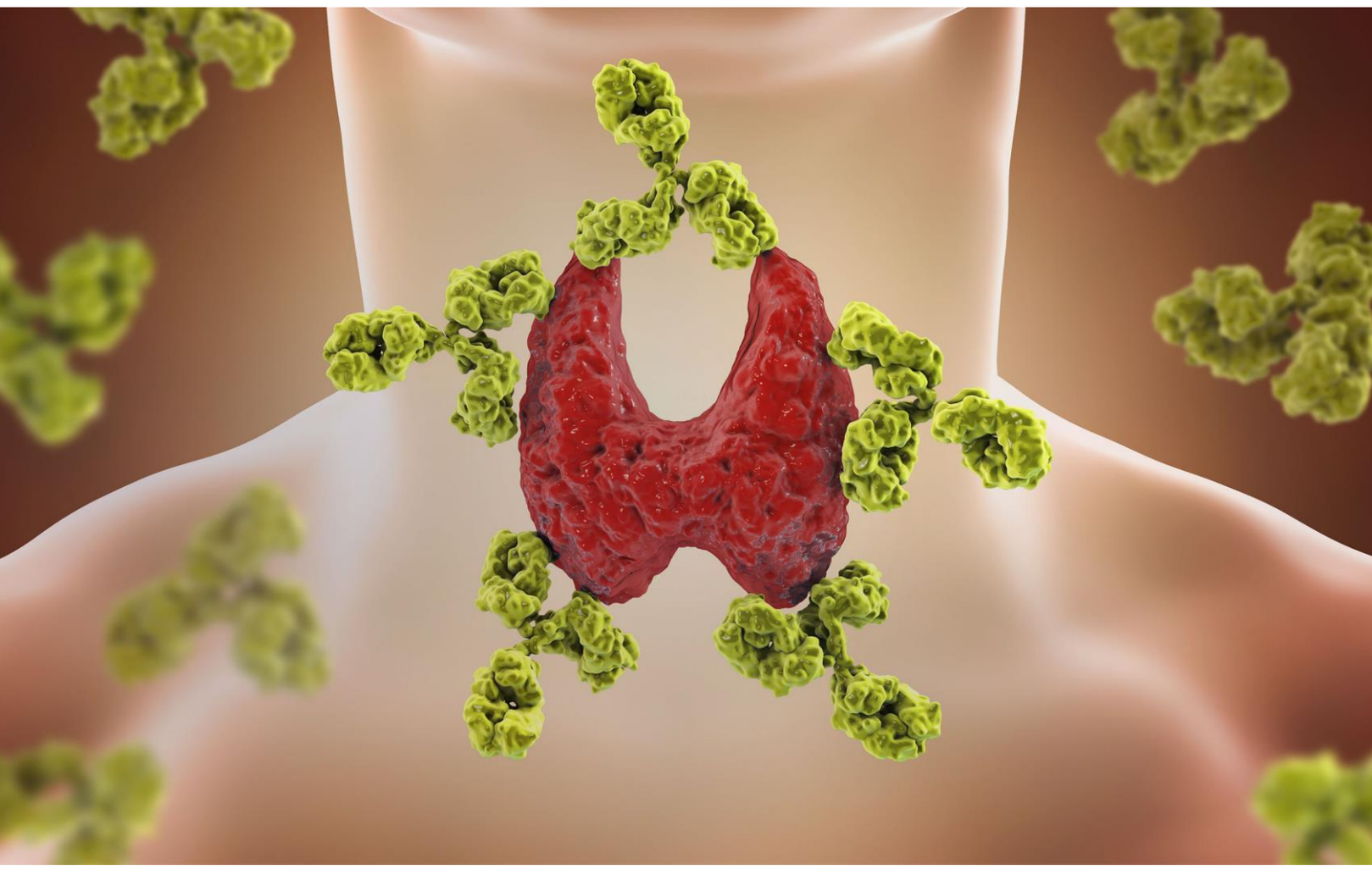
4. Furmaniak J.; *et al.* Blocking type TSH receptor antibodies. *Auto Immun Highlights.* 2012 Mar 21;4(1):11-26.

5. Chen CR.; *et al.* Crystal structure of a TSH receptor monoclonal antibody: insight into Graves' disease pathogenesis. *Mol Endocrinol.* 2015 Jan;29(1):99-107.

6. McLachlan SM, Rapoport B. Thyrotropin-blocking autoantibodies and thyroid-stimulating autoantibodies: potential mechanisms involved in the pendulum swinging from hypothyroidism to hyperthyroidism or vice versa. *Thyroid.* 2013 Jan;23(1):14-24.

CREATIVE DIAGNOSTICS

TSHR Antibodies for Graves' Disease Assay Development



Contact Us:

Tel: 1-631-624-4882 (USA) / 44-161-818-6441 (Europe)

Fax: 1-631-938-8221

Email: info@creative-diagnostics.com

www.creative-diagnostics.com

CD Creative Diagnostics®