

Human Recombinant Thyrotropin-releasing Hormone Receptor Stable Cell Line**Cat. No. M00202****Version 06092014**

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I. INTRODUCTION

Catalog Number: M00202

Cell Line Name: CHO-K1/TRH1

Gene Synonyms: MGC141920; TRHR

Expressed Gene: Genbank Accession Number NM_003301; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for TRH1 receptor

Freeze Medium: 45% culture medium, 45% FBS, and 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 400 μ g/ml G418

Mycoplasma Status: Negative

Storage Recommendation: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Thyrotropin-releasing hormone receptor1 (TRH1) is a member of G-protein coupled receptor family A. This protein is a receptor for Thyrotropin-releasing hormone (TRH). Human TRH1 is expressed in lymphocytes, pituitary gland and CNS. It can stimulate the releasing of prolactin (PRL), thyrotropin (TSH). TRH1 receptor knockout mice exhibit a slightly reduced growth rate, considerable decrease in serum T₃, T₄, and prolactin levels but no alteration of thyroid-stimulating hormone levels.

§: GenScript employs a PCR-based method to test the mycoplasma. The test covers 11 of the most common strains of mycoplasma, (covering approximately 95% of *M. fermentans*, *M. hyorhinis*, *M. arginini*, *M. orale*, *M. salivarium*, *M. hominis*, *M. pulmonis*, *M. arthritidis*, *M. neurolyticum*, *M. hyopneumoniae* and *M. capricolum*) and one species *Ureaplasma* (*U. urealyticum*), with sufficient sensitivity and specificity.

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III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by TRH in CHO-K1/TRH and CHO-K1 cells

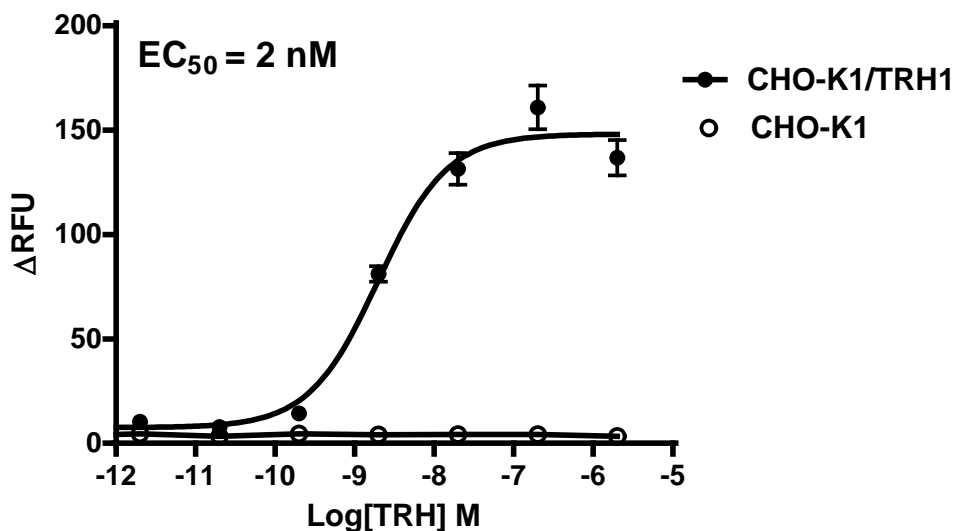


Figure 1. TRH-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/TRH1 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an TRH1 receptor agonist, TRH. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of TRH (Mean \pm SD, n = 2). The EC₅₀ of TRH on TRH1 in CHO-K1 cells was 2 nM. The S/B of TRH on TRH1 in CHO-K1 cells was 20.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{-(\text{LogEC}_{50} - X) \cdot \text{HillSlope}})$$

X is the logarithm of concentration. Y is the response
Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
- Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
- Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
- Resuspend the cells in complete growth medium.
- Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.

6. Grow the cells in incubator with 37°C, 5 %CO₂.
7. In the following day, replace the cells with fresh medium contains antibiotic.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).
Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Duthie SM (1993) Cloning and functional characterisation of the human TRH receptor. *Mol Cell Endocrinol.* Sep;95(1-2):R11-5
2. Cao J. Cloning and characterization of a cDNA encoding a novel subtype of rat thyrotropin-releasing hormone receptor. *J. Biol. Chem.*, 273, 32281 - 32287
3. Anderson L. Rapid desensitization of the thyrotropin-releasing hormone receptor expressed in single human embryonal kidney 293 cells. *Biochem J.*, 311, 385 - 392.

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