Human Recombinant Thyrotropin-releasing Hormone Receptor Stable Cell Line
Cat. No. M00202 **Version 06222020**

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
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for TRH1 receptor (Calcium flux assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 400 μg/ml G418 (Cat. #10131-035, Gibco)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt

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 T_3, T_4, and prolactin levels but no alteration of thyroid-stimulating hormone levels.

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

III. REPRESENTATIVE DATA

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

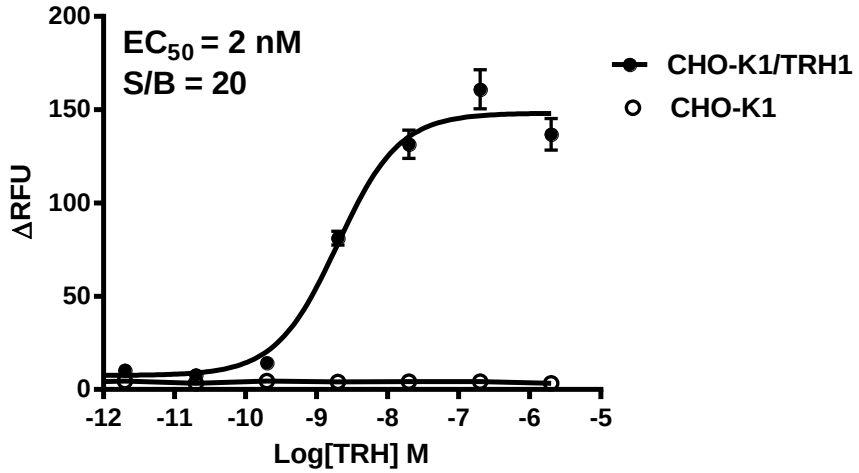

Figure 1: TRH-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/TRH1 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to being stimulated with a TRH1 receptor agonist, TRH. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (5-fold dilution) of TRH (Mean ± SD, n = 2). The EC_{50} of TRH on this cells was 2 nM.

Note:

1. EC_{50} value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((LogEC_{50}-X)*HillSlope))
   X is the logarithm of concentration. Y is the response and starts at Bottom and goes to Top with a sigmoid shape.
2. Signal to Background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. 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.
3. Pellet cells by centrifugation at 200 X g for 5 min, and remove the medium.
4. Resuspend the cells with 1 ml complete growth medium.
5. Transfer the cell suspension to a 10 cm dish containing 10 ml complete growth medium.
6. Transfer the dish into an incubator of 37°C, 5% CO_2.
7. Add antibiotic into the medium on the next day.

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 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 during incubation. 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 complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 X g for 5min, and remove the medium.
6. Resuspend the cells in culture medium and transfer the cells to a new culture dish.
7. Transfer the dish into an incubator of 37°C, 5% CO_2.

Subcultivation Ratio: 1:3 to 1:8
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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