Human Recombinant Calcitonin Receptor Stable Cell Line
Cat. No. M00320 **Version 07292020**

I. INTRODUCTION

Catalog Number: M00320
Cell Line Name: CHO-K1/CALCR/Gα15
Gene Synonyms: CALCR, CTR2, CT, CTRI1
Expressed Gene: Genbank Accession Number NM_001742; no expressed tags
Host Cell: CHO-K1/Gα15
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assay for CT 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, 100 μg/ml Hygromycin B (Cat. #10687010, Invitrogen), 200 μg/ml Zeocin (Cat. #R250-01, Life Technologies)
Mycoplasma Status: Negative*
Storage: Liquid nitrogen immediately upon receipt.

II. BACKGROUND

Numerous studies have demonstrated that the calcitonin receptor (CALCR) is a specific marker of osteoclast differentiation and that calcitonin can inhibit bone resorption *in vitro* and *in vivo*. Mice lacking calcitonin and calcitonin gene–related peptide (CGRP) have a high bone mass phenotype due to an increase in bone formation parameters. Expression of calcitonin (CT) and its receptor (CTR) generate survival, adhesion, pro-inflammatory, and pro-metastatic pathways. Moreover, data indicate a pivotal role for CT-CTR axis in advanced prostate cancer PC metastasis and may serve as a potential therapeutic target for advanced PC.

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

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Human Calcitonin in CHO-K1/CALCR/Gα15 cells

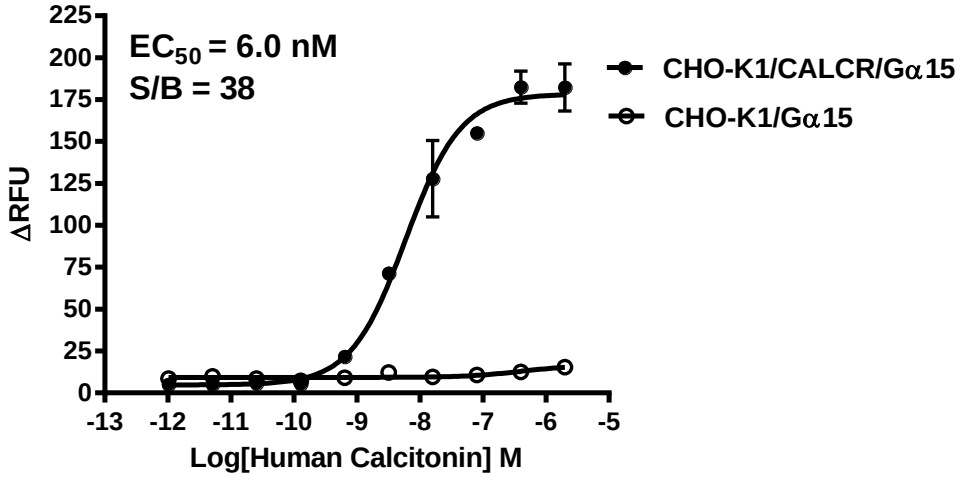

Figure 1. Human Calcitonin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CALCR/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist calcitonin. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of human calcitonin (Mean ± SD, n = 2). The EC_{50} of human calcitonin on this cell was 6.0 nM.

Notes:

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
   Y is RFU 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 force for 5 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. 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 %CO_2.
7. Add antibiotic in the following 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 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 %CO_2.

Subcultivation Ratio: 1:3 to 1:8
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Quinn, J.M., M. Morfis, M.H. Lam, *et al*. (1999) Calcitonin receptor antibodies in the identification of osteoclasts. *Bone*. 25:1–8.
2. Cornish, J., K.E. Callon, U. Bava, S.A. Kamona, *et al*. (2001) Effects of calcitonin, amylin, and calcitonin gene-related peptide on osteoclast development. *Bone*. 29:162–168.
3. Hoff, A.O., P. Catala-Lehnen, P.M. Thomas, M. Priemel, J.M. Rueger, *et al*. (2002) Increased bone mass is an unexpected phenotype associated with deletion of the calcitonin gene. *J. Clin. Invest*. 110:1849–1857.
4. Shah GV, Thomas S, Muralidharan A, *et al*. (2008) Calcitonin promotes in vivo metastasis of prostate cancer cells by altering cell signaling, adhesion, and inflammatory pathways. *Endocr Relat Cancer*. 15: 953-964

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