Human Recombinant Calcitonin Receptor Stable Cell Line

Technical Manual No. TM0510 **Version 06042010**

I. Introduction

Catalog Number: M00320

Cell Line Name: CHO-K1/CT/Gα15

Gene Synonyms: CALCR, CTR2, CT, CTRI1

Expressed Gene: Genbank Accession Number NM_001742.2; no expressed tags

Host Cell: CHO-K1/Gα15

Quantity: 2 vials (3×10^6 per vial) frozen cells

Stability: 16 passages

Application: Functional assay for CT receptor

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

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

Culture Medium: Ham's F12, 10% FBS, 100 μg/ml Hygromycin B, 200 μg/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery.

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.

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III. Representative Data

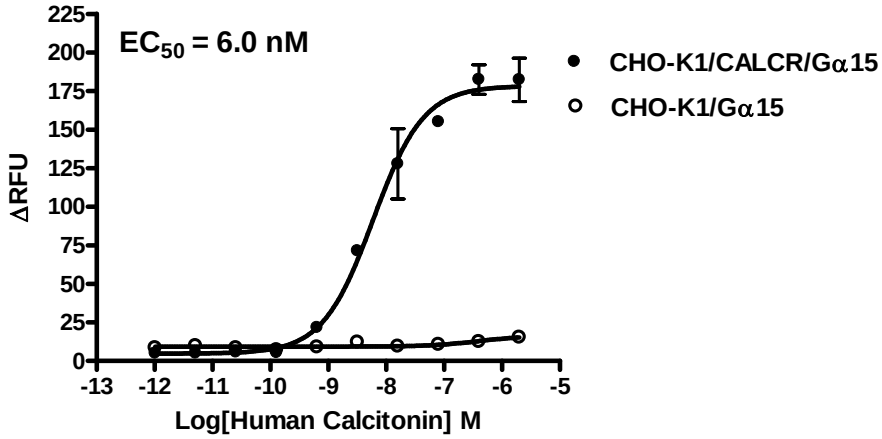

Figure Intracellular calcium response from CHO-K1/Gα15 cells stably expressing human calcitonin receptor CALCR and from untransfected control cells. Cells were loaded with Calcium-4 and then stimulated with the indicated concentrations of human calcitonin. Calcium responses were recorded on a FlexStation plate reader. Data represent the average +/- standard deviation of triplicate determinations.

IV. Thawing and Subculturing

Thawing: Protocol

1. Remove the vial from liquid nitrogen tank and thaw the 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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 2 ml of the cell suspension per well in a 6-well plate.
6. Add Hygromycin B and Zeocin to concentrations of 100 µg/ml and 200 µg/ml respectively the following day.

Subculturing: Protocol

1. Remove and discard culture medium.
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 to a 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g for 5min, and discard the medium.
5. Resuspend the cells in culture medium and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

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