Human Recombinant Parathyroid Hormone Receptor 1 Stable Cell Line

Technical Manual No. TM0507 **Version 10132010**

I. Introduction

Catalog Number: M00315

Cell Line Name: CHO-K1/PTH1/Gα15

Gene Synonyms: MGC138426; MGC138452; PFE; PTHR; PTHR1

Expressed Gene: Genbank Accession Number NM_000316; no expressed tags

Host Cell: CHO-K1/Gα15

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

Stability: 16 passages

Application: Functional assay for PTH1 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, 400 μg/ml G418

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery.

II. Background

Parathyroid hormone (PTH)/PTH-related peptide (PTHrP) receptor (PTHR1) is a G protein coupled receptor which mediates the actions of both amino-terminal PTH and PTHrP fragments. The most abundant expression of PTHR1 is found in renal tubular cells and in osteoblasts, where the PTH/PTHrP receptor mediates the endocrine actions of PTH, and in prehypertrophic chondrocytes of the metaphyseal growth plate, where it mediates the autocrine/paracrine actions of PTHrP. Intact PTH (PTH 1-84) is compounded by a peptide of 84 amino acids (AA), the amino-terminal sequence, constituted by the first 34 AA (N-terminal structure), is necessary for its action.

§: 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 PTH 1-34 in CHO-K1/PTH1/Gα15 and CHO-K1/Gα15 cells

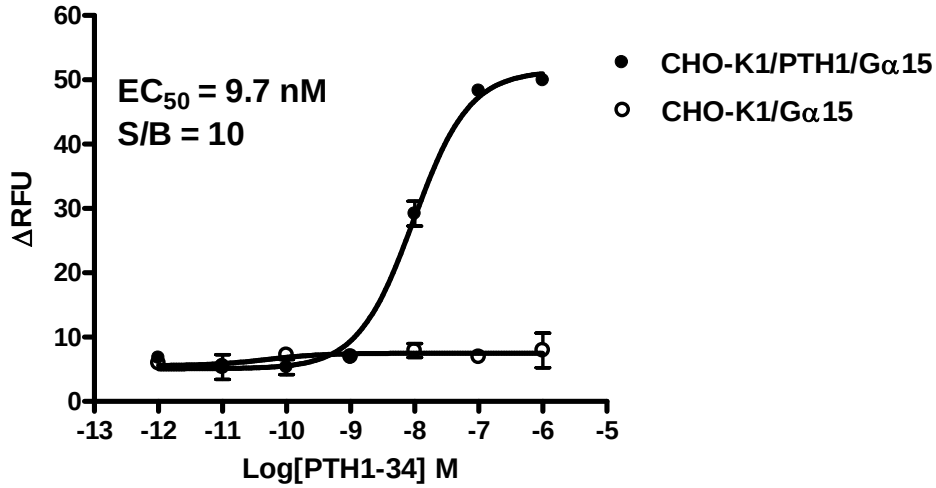

Figure 1. PTH 1-34-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/PTH/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with a PTH1 receptor agonist, PTH 1-34. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of PTH 1-34 (Mean ± SD, n = 2). The EC_{50} of PTH 1-34 on PTH1 co-expressing with Gα15 in CHO-K1 cells was 9.7 nM. The S/B of PTH 1-34 on PTH1 co-expressing with Gα15 in CHO-K1 cells was 10.

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.

Signal to background Ratio (S/B) = Top/Bottom

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 force for 5 min, and discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add Hygromycin B and G418 to concentrations of 100 µg/ml and 400 µg/ml respectively the following day.

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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 force 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.

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

V. References

1. C Leroy, D Manen1, R Rizzoli1, M Lombès2 and C Silve (2004) Functional importance of Myc-associated zinc finger protein for the human parathyroid hormone (PTH)/PTH-related peptide receptor-1 P2 promoter constitutive activity. *Journal of Molecular Endocrinology* 32, 99–113
2. C. de La Piedra, E. Fernández, M.ª L. González Casaus and E. González Parra (2008) Differences in the function of parathyroid peptides. What are we measuring? *Nefrología*; 28 (2) 123-128

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