

Human Recombinant Calcium Sensor Receptor Stable Cell Line

Technical Manual No. TM0561

Version 10132010

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I. Introduction

Catalog Number: M00434

Cell Line Name: CHO-K1/CASR

Gene Synonyms: CASR; CAR; FHH; FIH; GPRC2A; HHC; HHC1; MGC138441; NSHPT; PCAR1

Expressed Gene: Genbank Accession Number NM_000388; 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 CASR 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, 200 μ g/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

CASR is a calcium-sensing receptor and plays an important role in regulating PTH secretion. It is expressed in many different tissues, such as parathyroid, pancreatic islet and insulinoma cells, breast, gastrinoma cells, pituitary, keratinocytes and human colon epithelial cells. CASR Inhibits PTH secretion and parathyroid cellular proliferation in parathyroid glands or cells, and also inhibits proliferation of colonic crypt cells. Besides, it can stimulate PTHrP secretion in prostate cell line, and stimulate calcitonin secretion from thyroidal C-cells.

§: 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.

III. Representative Data

Concentration-dependent stimulation of intracellular calcium mobilization by CaCl_2 in CHO-K1/CASR and CHO-K1 cells

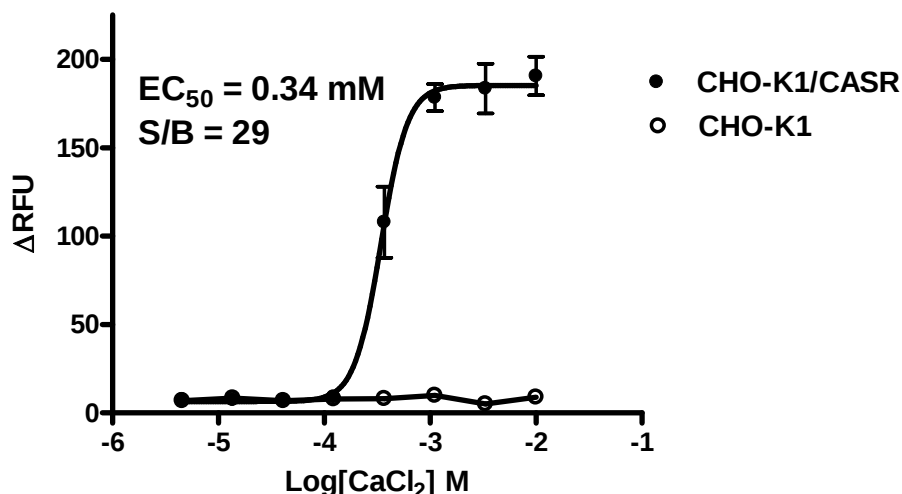


Figure 1. CaCl_2 -induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CASR and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a CASR receptor agonist, CaCl_2 . The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (3-fold dilution) of Histamine (Mean $\pm$ SD, $n = 2$). The EC_{50} of CaCl_2 on CASR in CHO-K1 cells was 0.34 mM. The S/B of CaCl_2 on CASR in CHO-K1 cells was 29.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:

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

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.
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 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 Zeocin to a concentration of 200 $\mu\text{g/ml}$ 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 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. Nemeth EF. (2004) Calcimimetic and calcilytic drugs: just for parathyroid cells? *Cell Calcium*. 35(3):283-9.
2. Sanders JL, (2001) Ca (2+)-sensing receptor expression and PTHrP secretion in PC-3 human prostate cancer cells. *Am J Physiol Endocrinol Metab*. 281(6):E1267-74.
3. Freichel M, (1996) Expression of a calcium-sensing receptor in a human medullary thyroid carcinoma cell line and its contribution to calcitonin secretion. *Endocrinology*. 137(9):3842-8.

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