Human Recombinant CCKB Cholecystokinin Receptor Stable Cell Line

Cat. No. M00154 **Version 05262014**

I. INTRODUCTION

Catalog Number: M00154

Cell Line Name: CHO-K1/CCKB

Expressed Gene: Genbank Accession Number NM_176875; no expressed tags

Gene Synonyms: CCKBR, CCKB

Host Cell: CHO-K1

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

Stability: 16 passages

Application: Functional assay for CCKB 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 μg/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Cholecystokinins (CCK) are derived from preprocholecystokinin. They are widely expressed in gastrointestinal tissues and in the CNS. There are two cholecystokinin receptors, referred to as A and B (CCKAR and CCKBR). CCKBR can be activated by the smaller CCK-4 (7, 8). In the CNS, CCKBR has been implicated in anxiety, depression, schizophrenia, depression, and opioid analgesia. In the stomach, CCK2 mediates gastrin-stimulated gastric acid secretion.

§: 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 CCK8 in CHO-K1/CCKB and CHO-K1 cells

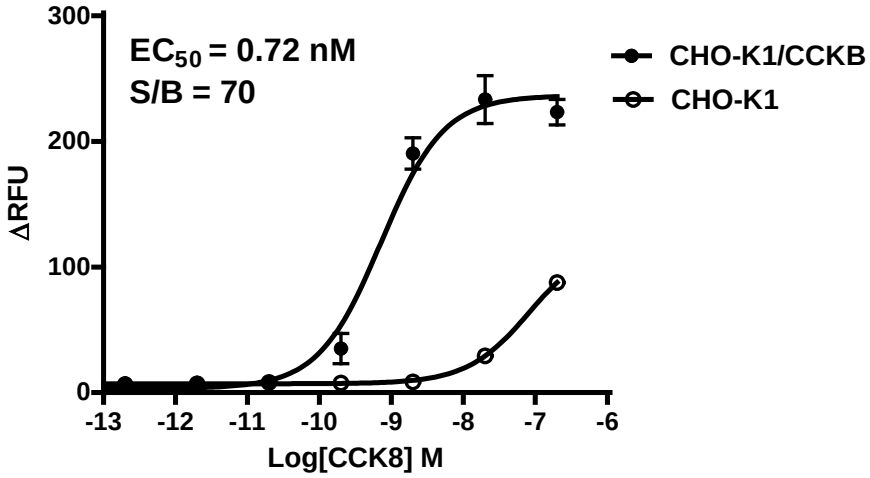

Figure 1. CCK8-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CCK8 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a CCKB receptor agonist, CCK8. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of CCK8 (Mean ± SD, n = 2). The EC_{50} of CCK8 on CCKB in CHO-K1 cells was 0.72 nM. The S/B of CCK8 on CCKB in CHO-K1 cells was 70.

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 weekly.
Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Noble, F. (1999) International Union of Pharmacology. Structure, distribution, and functions of cholecystokinin receptors. *Pharmacol Rev*, 51, 745 - 781.
2. Clerc, P. (2007) Involvement of cholecystokinin 2 receptor in food intake regulation: hyperphagia and increased fat deposition in cholecystokinin 2 receptor-deficient mice. *Endocrinology*, 148, 1039 - 1049.

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