Human Recombinant CCKA Cholecystokinin Receptor Stable Cell Line

Cat. No. M00271 **Version 05262014**

I. INTRODUCTION

Catalog Number: M00271

Cell Line Name: CHO-K1/CCKA/Gα15

Gene Synonyms: CCK1, CCKA, CCK-A

Expressed Gene: Genbank Accession Number NM_000730; 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 CCKA 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, 400 μg/ml G418, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Cholecystokinin (CCK)-A and CCK-B receptors are highly homologous members of the seven transmembrane domain G-protein-coupled receptor super-family. Peptides in the cholecystokinin (CCK) family have a variety of biological functions in the central and peripheral nervous systems as well as in the gastrointestinal tract. The CCKA receptor has a more limited distribution with the highest densities in the hypothalamic nuclei, areas of the hippocampus, the septum, dorsal motor vagal nucleus, and interpeduncular nucleus of the brain stem. It also occurs in numerous gastro-intestinal tissues. Binding of ligands to CCK1 stimulates mobilization of intracellular calcium by activation of Gq/11. CCKA receptors affect satiety, pancreatitis, and gut motility and have growth-promoting effects on some tumors.

§: 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 CCK-8 in CHO-K1/CCKA/Gα15 and CHO-K1 cells

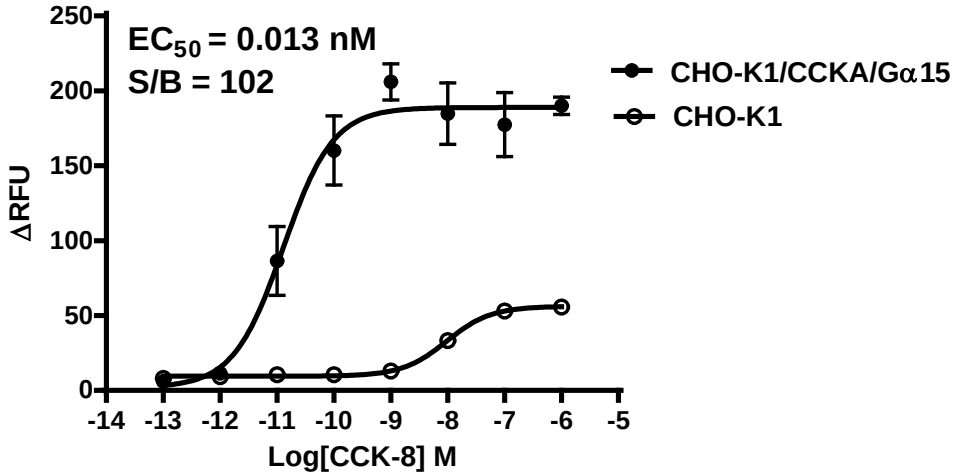

Figure 1. CCK-8-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/CCKA/Gα15 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a CCKA receptor agonist, CCK-8. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of CCK-8 (Mean ± SD, n = 2). The EC_{50} of CCK-8 on CCKA co-expressing with Gα15 in CHO-K1 cells was 0.013 nM. The S/B of CCK-8 on CCKA co-expressing with Gα15 in CHO-K1 cells was 102.

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. Vincent Wu, Moon Yang, James A. McRoberts, et al. (1997) First Intracellular Loop of the Human Cholecystokinin-A Receptor Is Essential for Cyclic AMP Signaling in Transfected HEK-293 Cells. *J. Biol. Chem*., *272: 9037-9042*.
2. Walsh, J. H. (1994) in Physiology of the Gastrointestinal Tract (Johnson, L. R., ed), *3rd Ed. pp. 1–128*.

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