Human Recombinant BB3 Bombesin Receptor Stable Cell Line

Cat.No.M00183 **05232014**

I. INTRODUCTION

Catalog Number: M00183

Cell Line Name: CHO-K1/BB3

Gene Synonyms: BRS3

Expressed Gene: Genbank Accession Number NM_001727; 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 BB3 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

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Bombesin, a bioactive peptide first identified in amphibian skin, is related to two mammalian peptides, gastrin-releasing peptide (GRP) and neuromedin B. A family of 4 GPCRs, including NMB-R (BB1), GRP-R (BB2), BRS-3 (BB3) and BRS-4, mediates the biological effects of the peptides. The bombesin receptor subtype 3, BB3 (also known as BRS3), is a third mammalian bombesin receptor subtype and no endogenous ligand has been identified to date. BB3 expression has been reported in lung (normal and cancer), nasal mucosa, placenta, and uterus. BB3-null mice have an obese phenotype, which suggests that BB3 may be an important target for obesity research. In addition, BB3 may be involved in diabetes and hypertension.

§: 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 Neuromedin B in CHO-K1/BB3 and CHO-K1 cells

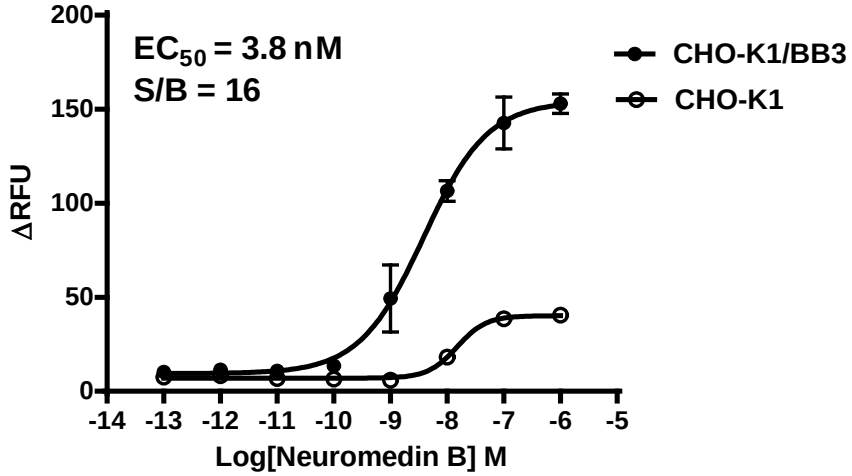

Figure 1. Neuromedin B-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/BB3 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a BB3 receptor agonist, Neuromedin B. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Neuromedin B (Mean ± SD, n = 2). The EC_{50} of Neuromedin B on BB3 in CHO-K1 cells was 3.8 nM. The S/B of Neuromedin B on BB3 in CHO-K1 cells was 21.

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. Schally AV, Comaru-Schally AM, Nagy A, *et al*. (2001) Hypothalamic hormones and cancer. *Front Neuroendocrinol* 22:248-91.
2. Sunday ME, Kaplan LM, Motoyama E, *et al*. (1988) Biology of disease, gastrin-releasing peptide (mammalian bombesin) gene expression in health and disease. *Lab Invest* 59:5-24.
3. Spindel ER, Giladi E, Segerson TP, Nagalla S. (1993) Bombesin-like peptides: of ligands and receptors. *Recent Progr Horm Res* 48:365-91.
4. Nagalla SR, Barry BJ, Creswick KC, *et al*. (1995) Cloning of a receptor for amphibian [Phe13]bombesin distinct from the receptor for gastrin-releasing peptide: identification of a fourth bombesin receptor subtype (BB4). *Proc Natl Acad Sci U S A* 92:6205-9.

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