Human Recombinant BB2 Bombesin Receptor Stable Cell Line

Cat. No. M00182 **Version 05232014**

I. INTRODUCTION

Catalog Number: M00182

Cell Line Name: CHO-K1/BB2

Gene Synonyms: GRPR

Expressed Gene: GenBank Accession Number NM_005314; no expressed tags

Host Cell: CHO-K1

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

Stability: 16 passages

Applications: Functional assays for BB2 receptors

Freeze Medium: 45% culture medium, 45% FBS, and 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 is a peptide isolated from the skins of various frogs. There are two peptides, neuromedin B (NMB) and gastrin-releasing peptide (GRP) that can be considered as its mammalian counterparts. The bombesin receptor family has been classified into three subtypes according to the affinity of each peptide to each receptor: BB1, BB2, and BB3. BB_2 receptor, which binds with GRP, is involved in a wide range of physiological functions. In the gastrointestinal tract, BB_2 receptor activation causes stimulation of acid secretion, pancreatic and intestinal secretion, and gastrointestinal motility. In CNS, it participates in regulating circadian rhythms, controlling body temperature, affecting grooming behaviors, and modulating fear, stress, anxiety, and memory. Additionally, BB_2 receptors are one of the most frequently and widely overexpressed and ectopically expressed G protein-coupled receptors in human tumors. BB_2 receptors may play a potential role in the treatment and management of these 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 Neuromedin B in CHO-K1/BB2 and CHO-K1 cells

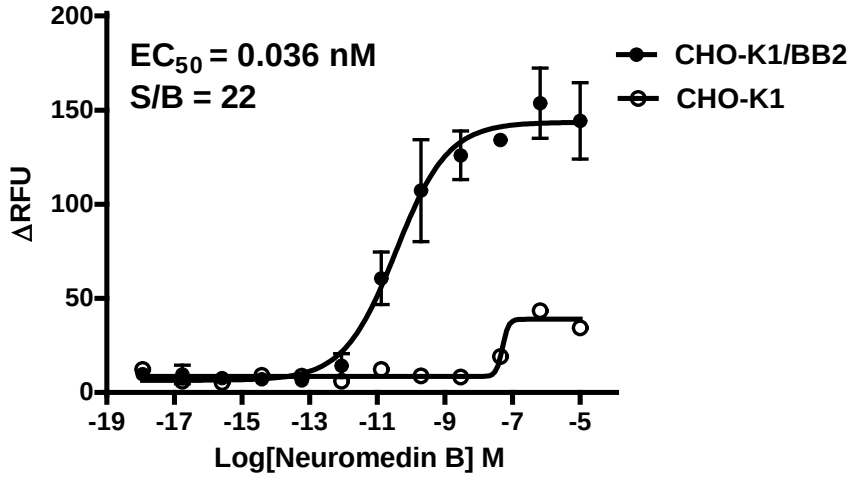

Figure 1. Neuromedin B-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/BB2 and CHO-K1. The cells were loaded with Calcium-4 prior to stimulation with a BB2 receptor agonist, Neuromedin B. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (15-fold dilution) of Neuromedin B (Mean ± SD, n = 2). The EC_{50} of Neuromedin B on BB2 in CHO-K1 cells was 0.036 nM. The S/B of Neuromedin B on BB2 in CHO-K1 cells was 22.

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. Corjay, M.H. (1991) Two distinct bombesin receptor subtypes are expressed and functional in human lung carcinoma cells. *J. Biol. Chem.* 266, 18771 - 18779.
2. Schubert, M.L. (1999) Regulation of gastric acid secretion. *Curr Opin Gastroenterol*, 15, 457 - 457.
3. Xiao, D. (2001) The human gastrin-releasing peptide receptor gene structure, its tissue expression and promoter. *Gene*, 264, 95 - 103.
4. Jensen, R.T. (2007) International Union of Pharmacology: The Bombesin Receptors. Nomenclature, distribution, pharmacology, signaling and functions in normal and diseased states. *Pharmacol. Rev.*, 60, 1 - 42.

GenScript USA Inc,
860 Centennial Ave.
Piscataway, NJ 08854
Toll-Free: 1-877-436-7274
Tel: 1-732-885-9188, Fax: 1-732-210-0262
Email: product@genscript.com
Web: http://www.genscript.com

For Research Use Only.

Limited Use License Agreement

This is a legal agreement between you (Licensee) and GenScript USA Inc. governing use of GenScript's stable cell line products and protocols provided to licensee. By purchasing and using the stable cell line, the buyer agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

1) The products are not transferable and will be used at the site where they were purchased. Transfer to another site owned by buyer will be permitted only upon written request by buyer followed by subsequent written approval by GenScript.
2) The purchaser cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party.
3) The products sold by GenScript are for laboratory and animal research purposes only. The products are not to be used on humans, for consumption, or for any unlawful uses.

GenScript USA Inc. will not assert against the buyer a claim of infringement of patents owned or controlled by GenScript USA Inc. and claiming this product based upon the manufacture, use or sale of a clinical diagnostic, therapeutic and vaccine, or prophylactic product developed in research by the buyer in which this product or its components has been employed, provided that neither this product nor any of its components was used in the manufacture of such product. For information on the use of this product for other purposes, contact Marketing Department, GenScript USA Inc., 120 Centennial Avenue, Piscataway, New Jersey 08840, U.S.A. Phone: 1-732-885-9188. Fax: 1-732-210-0262. Email: marketing@genscript.com.