# Human Recombinant BB1 Bombesin Receptor Stable Cell Line

**Cat. No. M00254** **Version 05232014**



## I. INTRODUCTION

Catalog Number: M00254

Cell Line Name: CHO-K1/BB1

Gene Synonyms: NMBR

Expressed Gene: Genbank Accession Number NM_002511; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells ($3\times10^6$ per vial)

Stability: 16 passages

Application: Functional assay for BB1 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 human BB1 receptor is a receptor for neuromedin-B (NMB), which is a potent mitogen and growth factor for normal and neoplastic lung and for gastrointestinal epithelial tissue. The NMBR pathway is involved in the regulation of a wide variety of behaviors, such as spontaneous activity, feeding and anxiety-related behavior. A study using NMBR-deficient mice suggested that dysfunction in the NMBR pathway may constitute one of the risk factors of stress vulnerability.

*§: 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/BB1 and CHO-K1 cells**

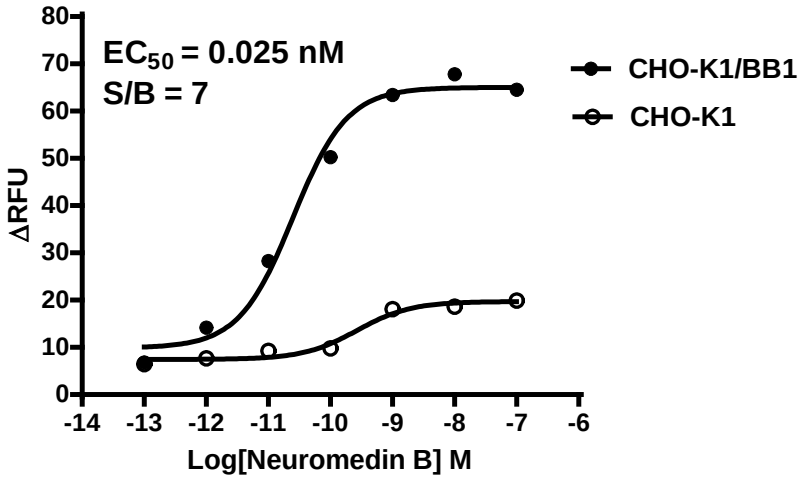


**Figure 1.** Neuromedin B-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/BB1 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with a BB1 receptor agonist, Neuromedin B. The intracellular calcium change was measured by FlexStation. 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 BB1 in CHO-K1 cells was 0.025 nM. The S/B of Neuromedin B on BB1 in CHO-K1 cells was 7.

**Notes:**

1. $EC_{50}$ value is calculated with four parameter logistic equation:
   Y=Bottom + (Top-Bottom)/(1+10^((Log$EC_{50}$-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. Benya, RV, *et al*. (1995). Expression and characterization of cloned human bombesin receptors. *Mol Pharmacol* 47(1): 10-20.
2. Moody, TW, *et al*. (2000). Nonpeptide neuromedin B receptor antagonists inhibit the proliferation of C6 cells. *Eur J Pharmacol* 409(2): 133-42.
3. Yamada, K, *et al*. (2002). Restraint stress impaired maternal behavior in female mice lacking the neuromedin B receptor (NMB-R) gene. *Neurosci Lett* 330(2): 163-6.

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