Human Recombinant NK3 Tachykinin Receptor Stable Cell Line

Cat. No. M00201 **Version 06092014**

I. INTRODUCTION

Catalog Number: M00201

Cell Line Name: CHO-K1/NK3

Gene Synonyms: TACR3, NK3R, TAC3RL, MGC148060, MGC148061

Expressed Gene: GenBank Accession Number NM 001059; 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 NK3 receptor

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

Tachykinins are peptides sharing a common C-terminal amino acid sequence: Phe-X-Gly-Leu-Met·NH_2. This neuropeptide family is composed of substance P, neurokinin A, and neurokinin B, which are widely distributed in mammalian central and peripheral nervous systems. These three molecules serve as both neurotransmitters and neuromodulators. Their actions are mediated by binding with three distinct receptors, namely NK1, NK2, and NK3. NK3 receptors show affinity for neurokinin B. They are predominantly expressed in both the peripheral and central nervous systems. NK3 receptors appear to modulate monoaminergic and amino acid neurotransmission. Studies show that manipulating modulation of NK3 receptor activity may have therapeutic utility in psychiatric diseases such as schizophrenia and affective disorders.

§: 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 Neurokinin B in CHO-K1/NK3 and CHO-K1 cells

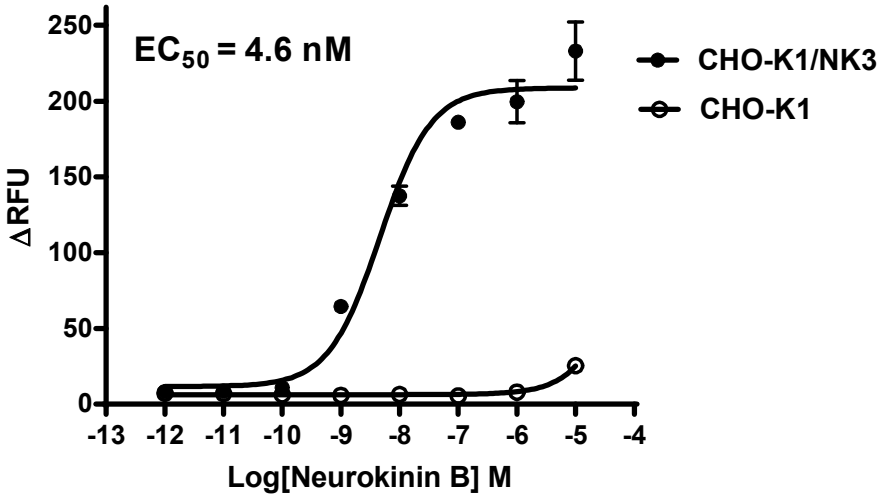

Figure 1. Neurokinin B-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NK3 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an NK3 receptor agonist, Neurokinin B. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Neurokinin B (Mean ± SD, n = 2). The EC_{50} of Neurokinin B on NK3 in CHO-K1 cells was 4.6 nM. The S/B of Neurokinin B on NK3 in CHO-K1 cells was 18.

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 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. In the following day, replace the cells with fresh medium contains antibiotic.

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. Huang, R.R. (1992) cDNA sequence and heterologous expression of the human neurokinin-3 receptor. *Biochem Biophys Res Commun*, 184, 966 - 972.
2. Dawson, L.A. (2008) *In vitro* and *in vivo* characterization of the non-peptide NK3 receptor antagonist SB-223412 (talnetant): potential therapeutic utility in the treatment of schizophrenia. *Neuropsychopharmacology.* 33(7):1642-52

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

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