Human Recombinant NK3 Tachykinin Receptor Stable Cell Line

Cat. No. M00201 **Version 07272020**

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
Culture Properties: Adherent
Quantity: Two vials of frozen cells (>1×10^6 per vial)
Stability: More than 16 passages
Application: Functional assays for NK3 receptor (Calcium flux assay, IP-One assay)
Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)
Complete Growth Medium: Ham's F-12 K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS
Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 100 µg/ml Hygromycin B (Cat. # 10687010, Invitrogen)
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.

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Neurokinin B in CHO-K1/NK3 cells

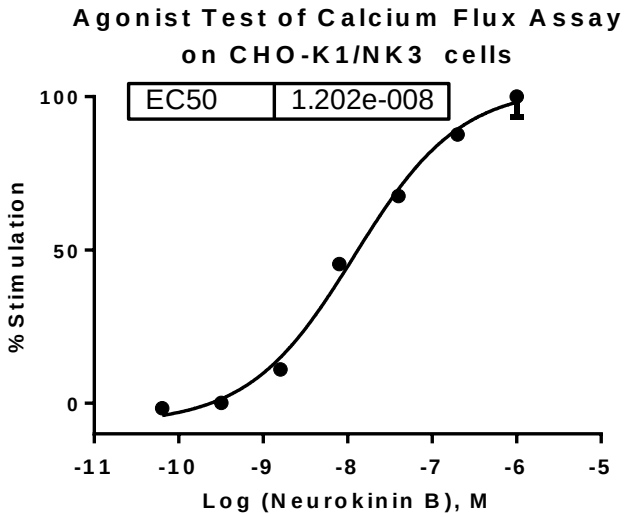

Figure 1: Neurokinin B-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NK3 cells. The cells were loaded with Calcium-4 prior to stimulation with an NK3 receptor agonist, Neurokinin B. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (5-fold dilution) of Neurokinin B (Mean ± SD, n = 2). The EC_{50} of Neurokinin B on NK3 in CHO-K1 cells was 12 nM.

Note:

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 and starts at Bottom and goes to Top with a sigmoid shape.

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 for 5 min, and remove the medium.
4. Resuspend the cells with 1 ml complete growth medium.
5. Transfer the cell suspension to a 10 cm dish containing 10 ml complete growth medium.
6. Transfer the dish into an incubator of 37°C, 5% CO_2.
7. Add antibiotic into the medium on the next 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 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 during incubation. 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 complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 X g for 5min, and remove the medium.
6. Resuspend the cells in culture medium and transfer the cells to a new culture dish.
7. Transfer the dish into an incubator of 37°C, 5% CO_2.

Subcultivation Ratio: 1:3 to 1:8
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

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