Human Recombinant NK1 Tachykinin Receptor Stable Cell Line

Cat. No. M00199 **Version 01112018**

I. INTRODUCTION

Catalog Number: M00199

Cell Line Name: CHO-K1/NK1

Gene Synonyms: TACR1, SPR, NK1, NKIR, TAC1R

Expressed Gene: GenBank Accession Number NM_001058; 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 NK1 receptors

Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO

Complete Growth Medium: Ham's F12, 10% FBS

Culture Media: Ham's F12, 10% FBS, 400 μg/ml G418

Mycoplasma Status: Negative[§]

Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Tachykinins are peptides sharing the 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. It plays a role as both a neurotransmitter and a neuromodulator. Their actions are mediated by binding with three distinct receptors, namely, NK1, NK2, and NK3. NK1 has high affinity with substance P. In the CNS, NK1 has been implicated to play a role in regulating neuronal survival and degeneration. In the cardiovascular system, NK1 mediates endothelium-dependent vasodilatation and plasma protein extravasations. In the gastrointestinal system, NK1 receptors mediate intestinal motility, secretion, and vascular functions. SP-NK1 receptor communication is also involved in glioma development and progression. NK_1 receptor antagonists may have several therapeutic applications in diseases mediated by tachykinins, such as pulmonary disorders, gut disorders, and the pathophysiology of depression.

§: 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 Substance P in CHO-K1/NK1 and CHO-K1 cells

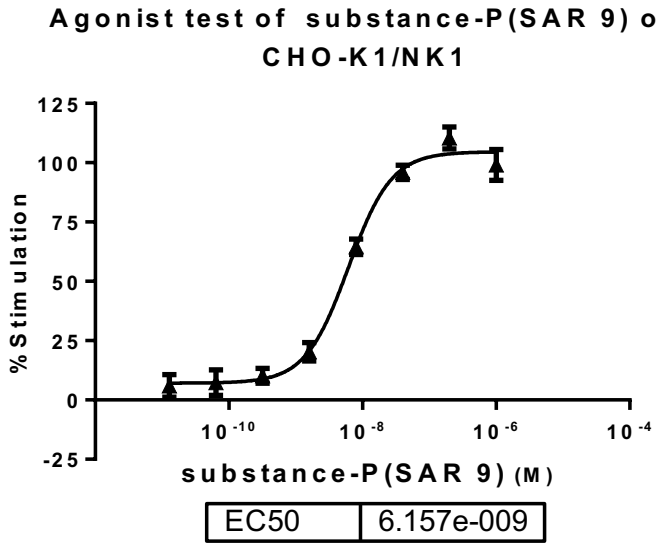

Figure 1. substance-P (SAR 9)-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NK1 cells. The cells were loaded with Calcium-4 prior to stimulation with an NK1 receptor agonist, substance-P (SAR 9). The intracellular calcium change was measured by FLIPR. The effects of agonist (%Stimulation) were plotted against the log of the cumulative doses (5-fold dilution) of substance-P (SAR 9) (Mean ± SD, n = 4). The EC50 of substance-P (SAR 9) on CHO-K1/NK1 in CHO-K1 cells was 6.15 nM. The S/B of substance-P (SAR 9) on NK1 in CHO-K1 cells was 7.5.

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. Quartara, L. (1998) The tachykinin NK1 receptor. Part II: Distribution and pathophysiological roles. *Neuropeptides.* 32(1):1-49
2. Deschamps, K. (2005) The ventral tegmental area as a putative target for tachykinins in cardiovascular regulation. *Br J Pharmacol.* 145(6):712-27

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