

Human Recombinant NK2 Tachykinin Receptor Stable Cell Line**Cat. No. M00200****Version 06092014**

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I. INTRODUCTION

Catalog Number: M00200

Cell Line Name: CHO-K1/NK2

Gene Synonyms: TACR2, SKR, NK2R, NKNAR, TAC2R

Expressed Gene: GenBank Accession Number NM001057; 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 NK2 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 μ g/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-NH₂. 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. In particular, NK2 is expressed in gastrointestinal tract. Activation of NK2 is chiefly responsible for the regulation of intestinal motor functions (both excitatory and inhibitory), secretions, inflammation, and visceral sensitivity. Antagonists of NK2 may be useful in the treatment of irritable bowel syndrome.

§: 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.

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III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Neurokinin A in CHO-K1/NK2 and CHO-K1 cells

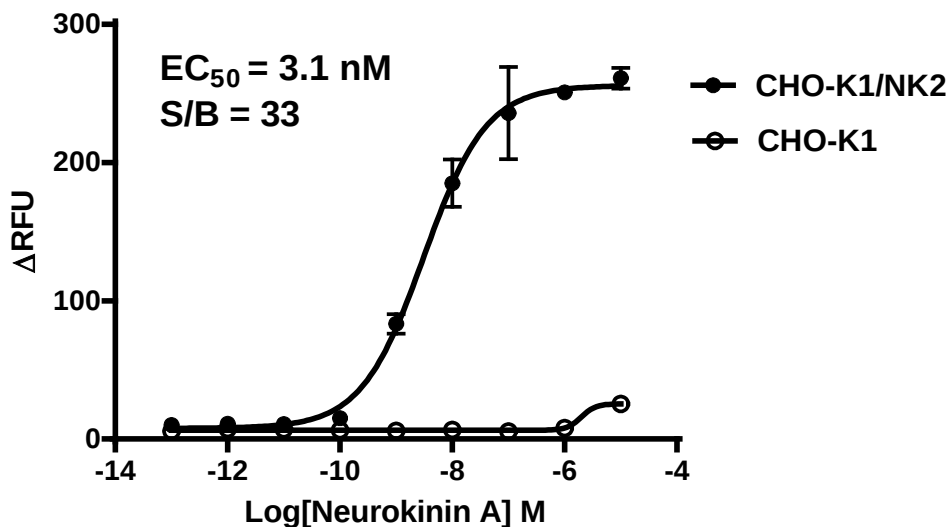


Figure 1. Neurokinin A-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NK2 and CHO-K1 cells. The cells were loaded with Calcium-4 prior to stimulation with an NK2 receptor agonist, Neurokinin A. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Neurokinin A (Mean $\pm$ SD, $n = 2$). The EC_{50} of Neurokinin A on NK2 in CHO-K1 cells was 3.1 nM. The S/B of Neurokinin A on NK2 in CHO-K1 cells was 33.

Notes:

1. EC_{50} value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{Log}EC_{50} - X) * \text{HillSlope})})$$

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 %CO₂.

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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 %CO₂.

Subcultivation Ratio: 1:3 to 1:8 weekly.

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. [Maggi, C.A.](#) (1992) Tachykinin antagonists inhibit nerve-mediated contractions in the circular muscle of the human ileum. Involvement of neurokinin-2 receptors. [Gastroenterology](#). 102(1):88-96
2. [Lecci, A.](#) (2004) Tachykinin NK2 receptor antagonists for the treatment of irritable bowel syndrome. [Br J Pharmacol](#). 141(8):1249-63
3. [Jaafari, N.](#) (2007) Distribution pattern of tachykinin NK2 receptors in human colon: involvement in the regulation of intestinal motility. [J Comp Neurol](#). 503(3):381-91

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