

Human Recombinant NK2 Tachykinin Receptor Stable Cell Line

Cat. No. M00200

Version 07272020

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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 NM_001057; no expressed tags

Host Cell: CHO-K1

Culture Properties: Adherent

Quantity: Two vials of frozen cells ($>1 \times 10^6$ per vial)

Stability: 16 passages

Application: Functional assays for NK2 receptor (Calcium flux assay)

Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)

Complete Growth Medium: Ham's F-12K (Kaighn's) (Cat. #21127, Life Technologies), 10% FBS

Culture Medium: Ham's F-12K (Kaighn's), 10% FBS, 400 μ g/ml G418 (Cat. #10131-035, Gibco)

Mycoplasma Status: Negative*

Storage: Liquid nitrogen immediately upon receipt

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. 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 A in

CHO-K1/NK2 cells:

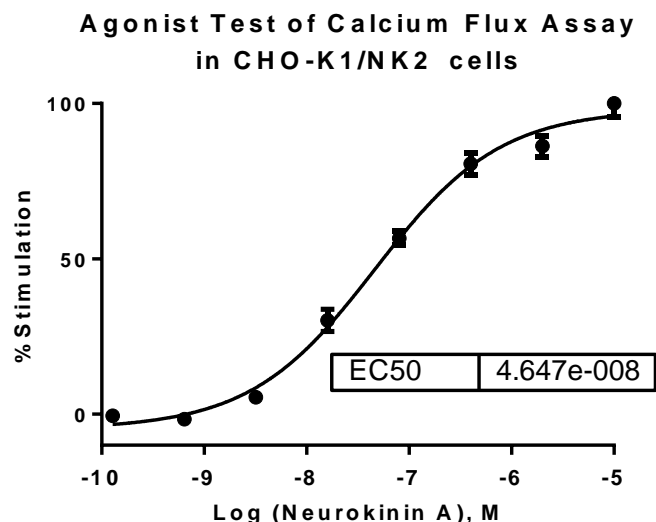


Figure 1: Neurokinin A-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NK2 cells. The cells were loaded with Calcium-4 prior to stimulation with an NK2 receptor agonist, Neurokinin A. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (5-fold dilution) of Neurokinin A (Mean \pm SD, n = 2). The EC₅₀ of Neurokinin A on this cells was 46.5 nM.

Note:

1. EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}$$
 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% CO₂.
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% CO₂.

Subcultivation Ratio: 1:3 to 1:8

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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