Human Recombinant NK1 Tachykinin Receptor Stable Cell Line

Technical Manual No. TM0395
Version 06042010

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 (3x10^6 per vial)
Stability: 16 passages
Applications: Functional assays for NK1 receptors
Freeze Medium: 45% culture medium, 45% FBS, 10% DMSO
Complete Culture 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

Tachyrhcinins are peptides sharing the 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. 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 vasodilation 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. NK1 receptor antagonists may have several therapeutic applications in diseases mediated by tachyrhchinins, such as pulmonary disorders, gut disorders, and the pathophysiology of depression.
III. Representative Data

**Figure:** Shown above are intracellular calcium responses from CHO-K1 cells stably expressing human NK1 tachykinin receptor and from untransfected control cells. Cells were loaded with Calcium-4 then stimulated with the indicated concentrations of substance P. Calcium responses were recorded on a FlexStation plate reader. Data represent the average +/- standard deviation of triplicate determinations.

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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 2 ml of the cell suspension per well in a 6 well-plate.
6. Add G418 to a concentration of 400 μg/ml the following day.

**Subculturing: Protocol**
1. Remove and discard culture medium.
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 to 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 clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting, centrifuge the cells 200 x g force for 5min, and discard the medium.
5. Resuspend the cells in complete growth medium with Zeocin and add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.
Subcultivation Ratio: 1:3 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V. References


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