Human Recombinant Neurotensin Receptor 1 Stable Cell Line

Cat. No. M00194 **Version 06092014**

I. INTRODUCTION

Catalog Number: M00194
Cell Line Name: CHO-K1/NTS1
Gene Synonyms: NTR; NTSR1
Expressed Gene: Genbank Accession Number NM_002531; no expressed tags
Host Cell: CHO-K1
Quantity: Two vials of frozen cells (3×10^6 per vial)
Stability: 16 passages
Application: functional assay for NTS1 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 μg/ml G418
Mycoplasma Status: Negative
Storage: Liquid nitrogen immediately upon delivery

II. BACKGROUND

Neurotensin receptor 1 (NTS1) is a member of G-protein coupled receptor family A and is a receptor for Neurotensin (NT). Neurotensin (NT) exerts its intracellular effect by interacting with 3 different receptors. Two of these receptors (NTR1 and NTR2) belong to the G protein-coupled receptor family, whereas the third one (NTR3) is a type I receptor with a single transmembrane domain. The NTS1 is expressed in CNS such as cerebral cortex, basal ganglia, limbic areas, vestibular system, and esophageal smooth muscle.

III. REPRESENTATIVE DATA

Concentration-dependent stimulation of intracellular calcium mobilization by Neurotensin in CHO-K1/NTS1 cells

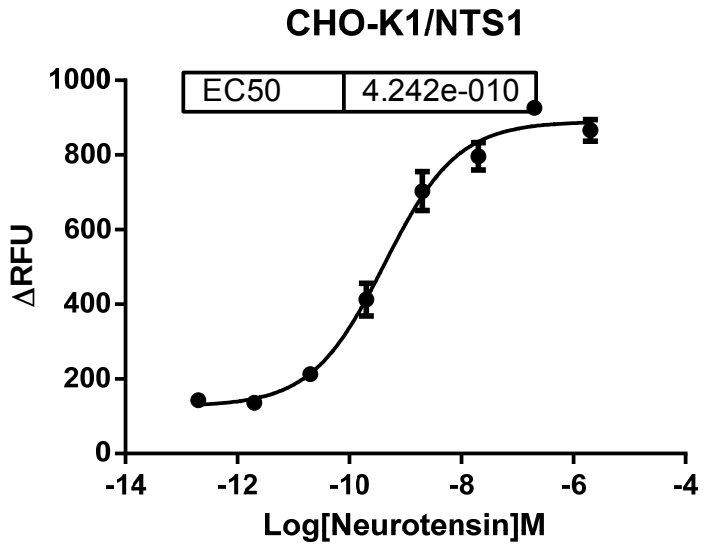

Figure 1. Neurotensin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NTS1 cells. The cells were loaded with Calcium-4 prior to stimulation with a NTS1 receptor agonist, Neurotensin. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of Neurotensin (Mean ± SD, n = 2). The EC_{50} of Neurotensin on NTS1 in CHO-K1 cells was 0.42 nM. The S/B of Neurotensin on NTS1 in CHO-K1 cells was 7.

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 the response
   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. Add antibiotic in the following 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 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

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