Human Recombinant Neurotensin Receptor 1 Stable Cell Line

Cat. No. M00194 **Version 06222020**

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

Culture Properties: Adherent

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

Stability: More than 16 passages

Application: functional assay for NTS1 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 μg/ml G418 (Cat. #10131-035, Gibco)

Mycoplasma Status: Negative*

Storage: Liquid nitrogen immediately upon receipt

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.

** The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.*

III. REPRESENTATIVE DATA

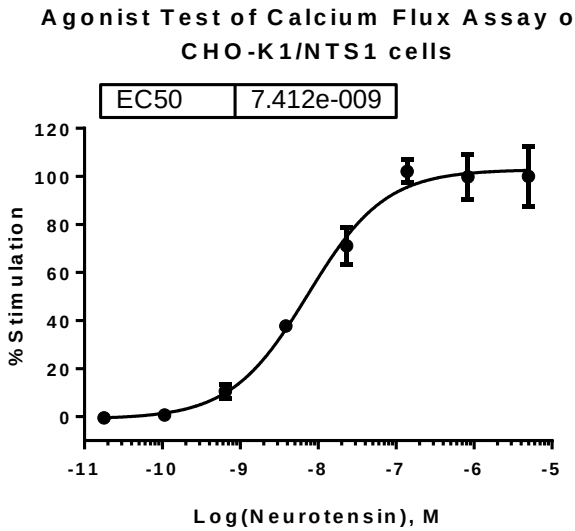

Figure 1: Neurotensin-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NTS1 cells. The cells were loaded with Calcium-4 prior to being stimulated with the agonist, neurotensin. The intracellular calcium change was measured by FLIPR^{TETRA}. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses of neurotensin (Mean ± SEM, n = 2). The EC_{50} of neurotensin on this cell was 7.4 nM.

Note:

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 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 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% CO_2.
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% CO_2.

Subcultivation Ratio: 1:3 to 1:8
Medium Renewal: Every 2 to 3 days

V. REFERENCES

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5. Choi,S.Y (1999). Characterization of high affinity neurotensin receptor NTR1 in HL-60 cells and its down regulation during granulocytic differentiation. *Br J Pharmacol*, 126, 1050 - 1056.
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