Human Recombinant Neuromedin U Receptor 2 Stable Cell Line

Technical Manual No. TM0586 **Version 10132010**

I. Introduction

Catalog Number: M00244

Cell Line Name: 293T/NMU2

Gene Synonyms: NMUR2; FM4; NMU2R

Expressed Gene: Genbank Accession Number NM_020167; no expressed tags

Host Cell: 293T

Quantity: Two vials of frozen cells (3×10^6 per vial)

Stability: 16 passages

Application: Functional assay for NMU2 receptor

Freeze Medium: 45% culture medium, 45% FBS, and 10% DMSO

Complete Growth Medium: EMEM, 10% FBS

Culture Medium: DMEM, 10% FBS, 100 µg/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

NMU1 and NMU2 are two G-protein coupled receptors binding to Neuromedin U (NmU) that is a peptide which regulates peripheral functions such as smooth muscle contraction and blood pressure, and CNS functions including nociception and feeding activity. Compared to the wide distribution of NMU1 in peripheral tissue, expression of NMU2 receptor is limited to areas of the brain, such as the paraventricular nucleus, along the wall of the third ventricle in the hypothalamus and the CA1 region of the hippocampus, and to the spinal cord.

§: 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 NMU25 in 293T/NMU2 and 293T cells

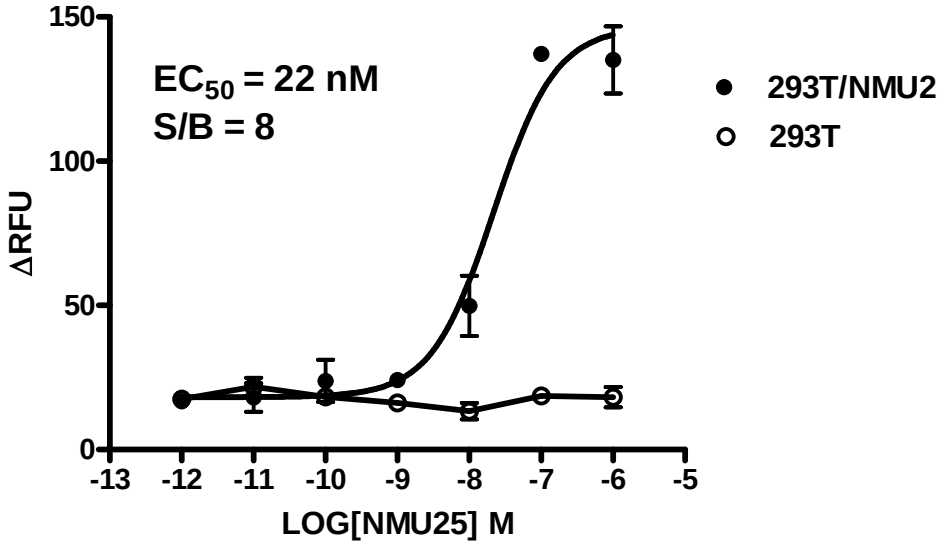

Figure 1. NMU25-induced concentration-dependent stimulation of intracellular calcium mobilization in 293T/NMU2 and 293T cells. The cells were loaded with Calcium-4 prior to stimulation with a NMU2 receptor agonist, NMU25. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of NMU25 (Mean ± SD, n = 2). The EC_{50} of NMU25 on NMU2 in 293T cells was 22 nM. The S/B of NMU25 on NMU2 in 293T cells was 8.

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 discard the medium.
4. Resuspend the cells in complete growth medium.
5. Add 10 ml of the cell suspension in a 10 cm dish.
6. Add Zeocin to a concentration of 100 µg/ml the following day.

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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 culture medium 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

1. LiXin Shan (2000). Identification of a novel neuromedin U receptor subtype expressed in the central nervous system. *The Journal of Biological Chemistry.* Vol.275 No.50: 39482–39486.
2. Raddadz R,Wilson (2000). Identification and characterization of two neuromedin U receptors differentially expressed in peripheral tissues and the central nervous system. *J. Biol. Chem.* 275, 32452 - 32459

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