Human Recombinant Neuropeptide S Receptor Isoform B Stable Cell Line

Technical Manual No. TM0549 **Version 10132010**

I. Introduction

Catalog Number: M00344

Cell Line Name: CHO-K1/NPS1b/Gα15

Expressed Gene: Genbank Accession Number NM_207173; no expressed tags

Host Cell: CHO-K1/Gα15

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

Stability: 16 passages

Application: Functional assay for NPS1b receptor

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

Complete Growth Medium: Ham's F12, 10% FBS

Culture Medium: Ham's F12, 10% FBS, 200 μg/ml Zeocin, 100 μg/ml Hygromycin B

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

The NPS receptor is a typical GPCR, also known as GPR154, vasopressin-receptor related receptor 1 (VRR1), or GPRA. NPSR was found mainly expressed in the central nervous system of rats by using *in-situ* hybridization. NPS receptor mRNA is widely distributed in many brain areas with high expression levels in cortex, hypothalamus, amygdala and multiple midline thalamic nuclei. Many of these areas have been functionally associated with arousal and processing of emotional behavior. In 2004, the NPS receptor was identified as an asthma susceptibility gene in a genome wide screen in Finnish and Canadian patients. The study showed that a number of polymorphic variants of the NPS receptor exist in human and that particular sets of these variants (haplotypes) are associated with an increased risk of asthma and possibly allergic diseases characterized by high IgE serum levels. A carboxy-terminal splice variant of human NPS receptor was found to be over-expressed in asthmatic airway tissue. Expression of NPS receptor mRNA was also found upregulated in a mouse model of airway inflammation.

§: 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 NPS in CHO-K1/ NPS1b/Gα15 and CHO-K1/Gα15 cells

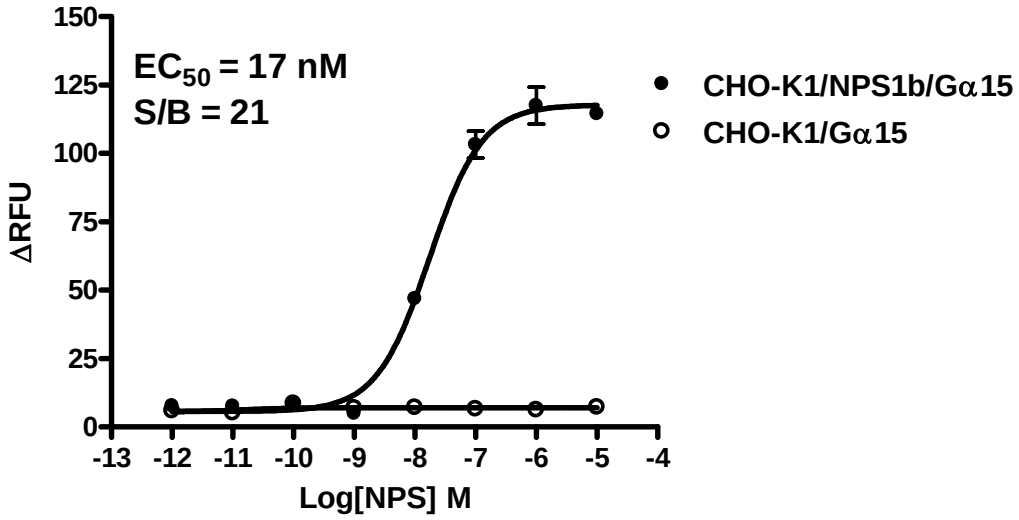

Figure 1. NPS-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NPS1b/Gα15 and CHO-K1/Gα15 cells. The cells were loaded with Calcium-4 prior to stimulation with an NPS1b receptor agonist, NPS. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of NPS (Mean ± SD, n = 2). The EC_{50} of NPS on NPS1b co-expressing with Gα15 in CHO-K1 cells was 17 nM. The S/B of NPS on NPS1b co-expressing with Gα15 in CHO-K1 cells was 21.

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 Hygromycin B and Zeocin to concentrations of 100 μg/ml and 200 μg/ml respectively 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. Gupte, J., Cutler, G., Chen, *et al.* (2004) Elucidation of signaling properties of vasopressin receptor-related receptor 1 by using the chimeric receptor approach. *Proc. Natl. Acad. Sci. U.S.A.*. 101: 1508-1513
2. Laitinen, T., Polvi, A., Rydman, P., *et al.* (2004) Characterization of a common susceptibility locus for asthma-related traits. *Science*. 304: 300-304
3. Xu, Y. L., Reinscheid, R. K., Huitron-Resendiz, S., *et al*. (2004) Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. *Neuron.* 43: 487-497
4. Xu, Y. L., Gall, C. M., Jackson, V. R., *et al.* (2007) Distribution of neuropeptide S receptor mRNA and neurochemical characteristics of neuropeptide S-expressing neurons in the rat brain. *J Comp Neurol,* 500: 84-102

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