Human Recombinant Neuropeptide Y Receptor Y2 Stable Cell Line

Technical Manual No. TM0627 **Version 05092011**

I. Introduction

Catalog Number: M00460

Cell Line Name: CHO-K1/NPY2/Gqi5

Gene Synonyms: NPY2R

Expressed Gene: Genbank Accession Number NM_000910; no expressed tags

Host Cell: CHO-K1/Gqi5

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

Stability: 16 passages

Application: Functional assay for NPY2 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, 100 µg/ml Hygromycin B, 200 µg/ml Zeocin

Mycoplasma Status: Negative

Storage: Liquid nitrogen immediately upon delivery

II. Background

The NPY family consists of three 36-amino acid peptides, neuropeptide Y (NPY), peptide YY (PYY) and pancreatic polypeptide (PP), which bind to the NPY receptors. At present five distinct NPY receptors, Y1, Y2, Y4, Y5, and y6, have been established by receptor cloning studies and all of them are G_i-coupled GPCRs. Activation of NPY receptors mediate a variety physiological effects including stimulation of food intake, inhibition of anxiety in the CNS, presynaptic inhibition of neurotransmitter release in the CNS and periphery, modulation of circadian rhythm, release of pituitary hormones, modulation of hippocampal activity, pain transmission, vasoconstriction, inhibition of insulin release and modulation of renal function. With regard to endogenous agonists, the receptors Y2 preferentially bind NPY and PYY.

§: 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 NPY in CHO-K1/NPY2/Gqi5 and CHO-K1/Gqi5 cells

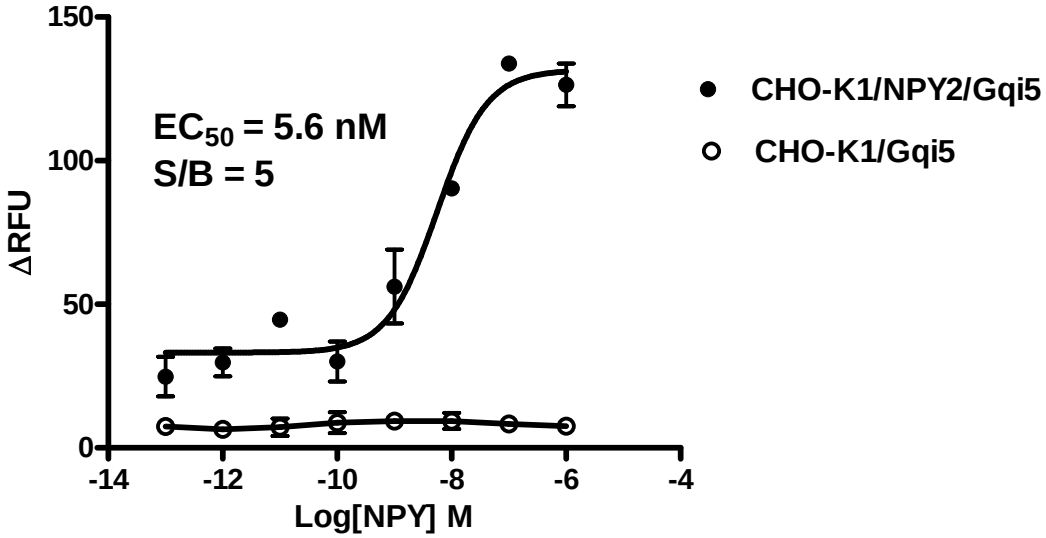

Figure 1. NPY-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NPY2/Gqi5 and CHO-K1/Gqi5 cells. The cells were loaded with Calcium-4 prior to stimulation with a NPY2 receptor agonist, NPY. The intracellular calcium change was measured by FlexStation. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (10-fold dilution) of NPY (Mean ± SD, n = 2). The EC_{50} of NPY on NPY2 co-expressing with Gqi5 in CHO-K1 cells was 5.6 nM. The S/B of NPY on NPY2 co-expressing with Gqi5 in CHO-K1 cells was 5.

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. Misra S, *et al.* (2004) Coexpression of Y1, Y2, and Y4 receptors in smooth muscle coupled to distinct signaling pathways. *J Pharmacol Exp Ther.* 311(3):1154-62. Epub Aug 12.
2. Lundell I, *et al.* (1995) Cloning of a human receptor of the NPY receptor family with high affinity for pancreatic polypeptide and peptide YY. *J Biol Chem.* 270(49):29123-8.

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