
Human Recombinant Neuropeptide Y Receptor NPY4 Stable Cell Line

Cat. No. M00237

Version 06282020

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I. INTRODUCTION

Catalog Number: M00237

Cell Line Name: CHO-K1/NPY4/Gα15

Gene Synonyms: NPY4R

Expressed Gene: NM_005972 (no expressed tags)

Host Cell: CHO-K1/Gα15

Culture Properties: Adherent

Quantity: Two vials of frozen cells (>1×10⁶ per vial)

Stability: More than 16 passages

Application: Functional assay for NPY4R receptor (calcium flux assay)

Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)

Culture Medium: Ham's F-12K (Kaighn's) (Cat. #11765, Life Technologies), 10% FBS, 400 µg/ml G418 (Cat. #10131-035, Gibco), 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen)

Mycoplasma Status: Negative*

Storage: Liquid nitrogen immediately upon receipt

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. Y4 receptor preferentially binds PP, with significant binding to NPY and PYY.

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

III. REPRESENTATIVE DATA

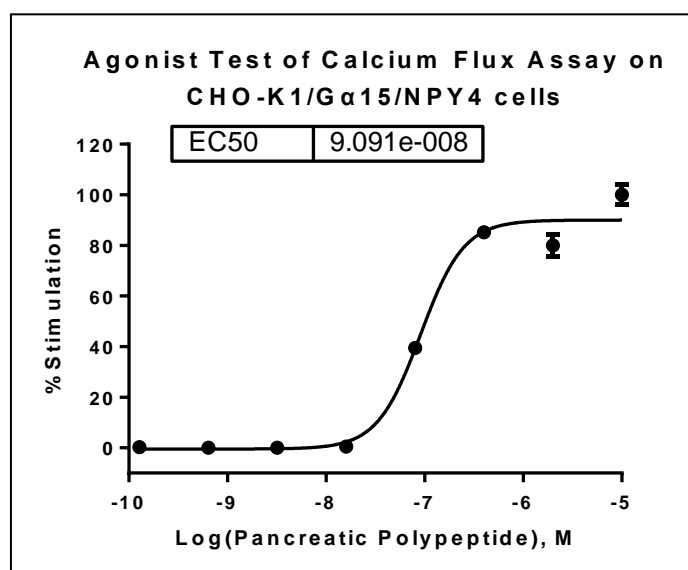


Figure 1: Pancreatic Polypeptide-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NPY4/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with a NPY4 receptor agonist, pancreatic polypeptide. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were normalized and plotted against the log of the cumulative doses (5-fold dilution) of pancreatic polypeptide (Mean ± SD, n = 2). The EC₅₀ of pancreatic polypeptide on this cell was 91 nM.

Note:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + (\text{Top} - \text{Bottom}) / (1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope})})$$
 X is the logarithm of concentration. Y is the response and starts at Bottom and goes to Top with a sigmoid shape.
- Signal to Background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- 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.
- Pellet cells by centrifugation at 200 X g for 5 min, and remove the medium.
- Resuspend the cells with 1 ml complete growth medium.
- Transfer the cell suspension to a 10 cm dish containing 10 ml complete growth medium.
- Transfer the dish into an incubator of 37°C, 5% CO₂.
- 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% CO₂.

Subcultivation Ratio: 1:3 to 1:8

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.
3. Voisin T, et al. (2000) Functional and molecular properties of the human recombinant Y4 receptor: resistance to agonist-promoted desensitization. *J Pharmacol Exp Ther.* 292(2):638-46.

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