

Neuropeptide Y Receptor Y2 (NPY2R) ACTOne™ Stable Cell Line

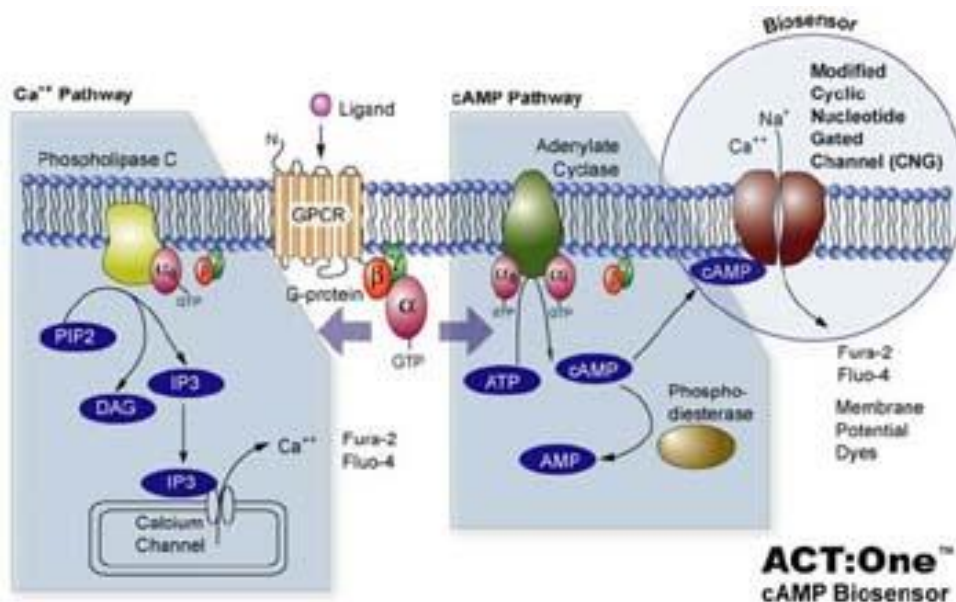
CATALOG NUMBER: CL-11-NPY2R

Introduction

Neuropeptide Y (NPY) receptors are a family of Gi/o-protein-coupled receptors that are currently divided into four subtypes: Y1, Y2, Y4 and Y5. NPY receptors mediate a diverse range of biological actions including stimulation of food intake, anxiolysis, modulation of circadian rhythm, pain transmission and control of pituitary hormone release. All four NPY receptor subtypes are found at high concentrations in the brain and colon and are also found in a wide variety of peripheral tissues at lower levels.

Description

Human NPY2R ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human NPY2R. HEK-293 CNG cells express a modified CNG (Cyclic Nucleotide Gated) channel that opens in response to elevated intracellular cAMP levels and consequently result in ion flux (often detectable by calcium-responsive dye, Cat# CA-C155) and cell membrane depolarization which can be easily measured with fluorescent Membrane Potential Dye (Cat# CA-M165). The assay allows both end-point and kinetic measurement of intracellular cAMP changes with a FLIPR, or a fluorescence microplate reader.



Parental Cells

HEK-293 CNG cells (originally developed by BD Biosciences by introducing CNG in HEK-293 cells) (Cat# CL-03-PC20)

Gene/Enzyme Introduced

NPY2R (Genbank Accession No. NP_000901.1)

Applications

- cAMP dependent assay for Gi-coupled human NPY2R
- cell based high-throughput screening of human NPY2R inhibitors

Functional Test

- this cell line has been tested positive for NPY2R specific response

- surviving rate: More than 2.5 million/vial on the second day after thawing
- the receptor specific activity is stable for 10 weeks continuous passage

Mycoplasma Contamination Test

This lot of cells has been tested and found to be free of mycoplasma contamination.

Content

- Stable cells: 1 mL (1×10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250 μ g/ml G418, 1 μ g/ml Puromycin
- Freezing medium: 10% DMSO, 90% complete cell culture medium

Storage

Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C , preferably in liquid nitrogen vapor, until ready for use.

Data Analysis

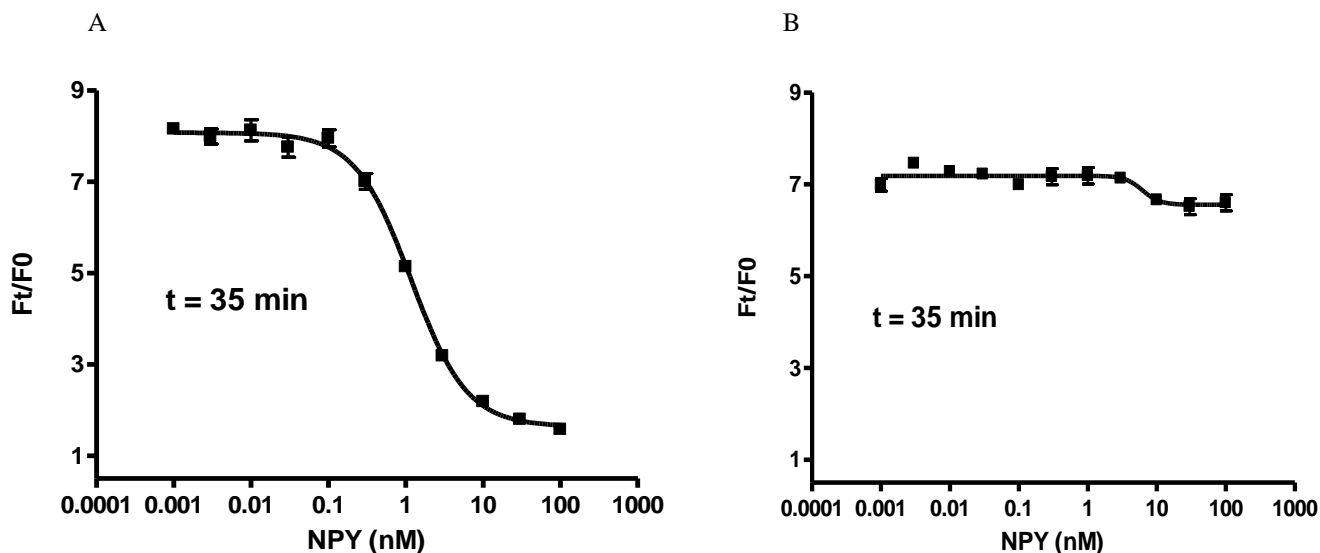


Figure 1. Response of ACTOne™ NPY2R cell line & parental cell line to NPY.

ACTOne™ NPY2R cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20 μ l culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 μ l/well of 1x Dye-loading solution (membrane potential dye kit, Cat# CA-M165). After 2 hours of incubation at room temperature, two readings were obtained prior to and 30 min after the addition of NPY. Ratios of the two readings (F/F0) are plotted in the figure.

- Dose response curve of NPY in ACTOne™ NPY2R cell line. EC50 = 1.2 nM in the presence of PDE inhibitor Ro 20-1724 and β -adrenoceptor agonist isoproterenol.**
- Parental cells do not respond to NPY.**

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