

## Neuropeptide Y Receptor Y1 (NPY1R) ACTOne™ Stable Cell Line

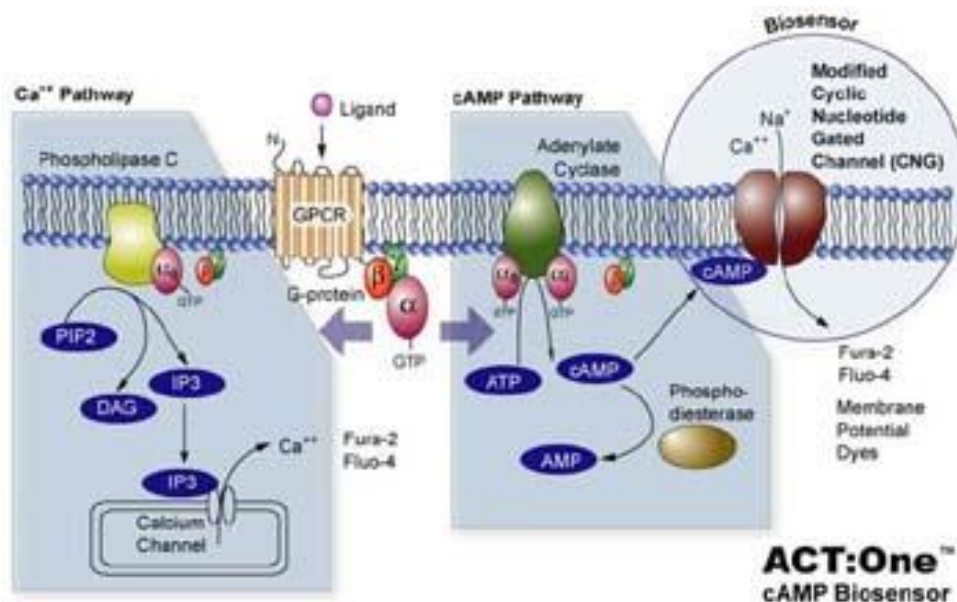
CATALOG NUMBER: CL-11-NPY1R

### 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. NPY1R mediates the function of neuropeptide Y (NPY), a neurotransmitter, and peptide YY (PYY), a gastrointestinal hormone. The encoded receptor undergoes fast agonist-induced internalization through clathrin-coated pits and is subsequently recycled back to the cell membrane. Activation of Y1 receptors may result in mobilization of intracellular calcium and inhibition of adenylate cyclase activity.

### Description

Human NPY1R ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human NPY1R. 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

NPY1R (Genbank Accession No. NP\_000900.1)

### Applications

- cAMP dependent assay for Gi-coupled human NPY1R
- cell based high-throughput screening of human NPY1R inhibitors

### Functional Test

- this cell line has been tested positive for NPY1R 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 ( $1 \times 10^6$  cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

### Growth Properties

Adherent

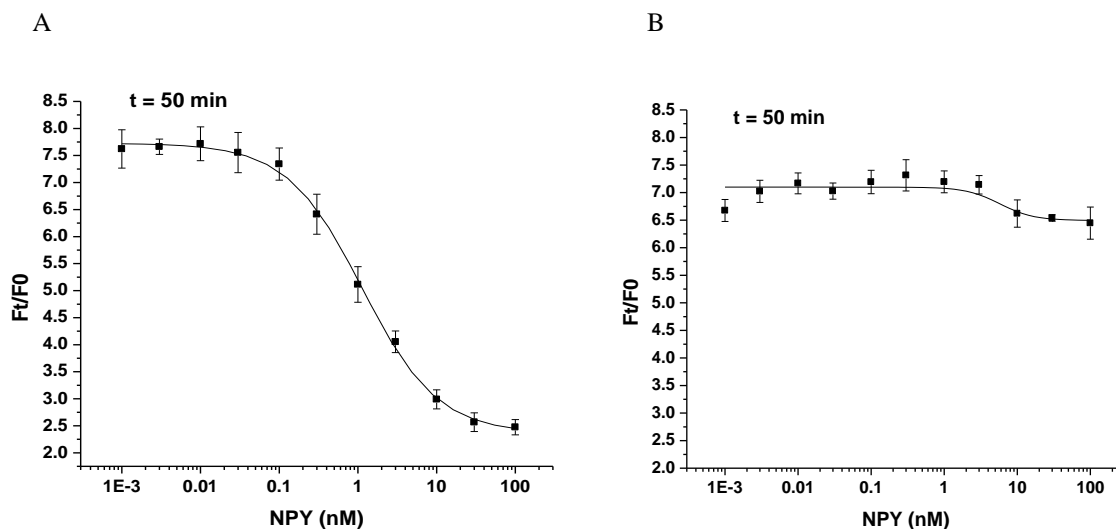
### Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250  $\mu$ g/ml G418, 1  $\mu$ 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  $-130^{\circ}\text{C}$ , preferably in liquid nitrogen vapor, until ready for use.

### Data Analysis



**Figure 1. Response of ACTOne™ NPY1R cell line & parental cell line to NPY.**

ACTOne™ NPY1R cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20  $\mu$ l culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20  $\mu$ 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™ NPY1R cell line. EC50 = 1.14 nM in the presence of PDE inhibitor Ro 20-1724 and  $\beta$ -adrenoceptor agonist isoproterenol.**
- Parental cells do not respond to NPY.**

### Notice to Purchaser

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