

Parathyroid Hormone 2 Receptor (PTH2R) ACTOne™ Stable Cell Line

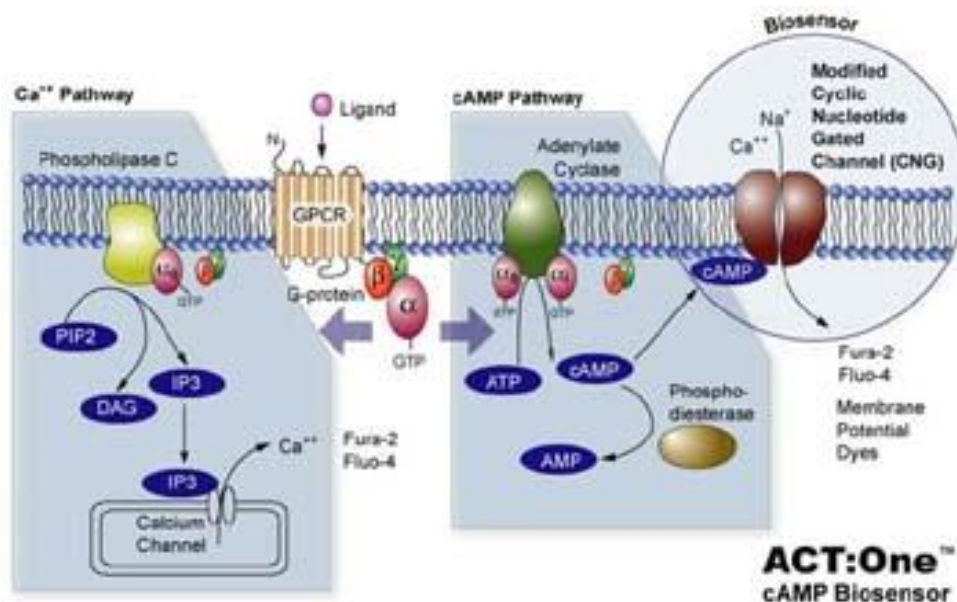
CATALOG NUMBER: CL-01-PTH2R

Introduction

PTH2R is a member of the G protein-coupled receptor family 2. This protein is a receptor for parathyroid hormone (PTH). This receptor is more selective in ligand recognition and has a more specific tissue distribution compared to parathyroid hormone 1 receptor (PTH1R). It is activated only by PTH and not by parathyroid hormone-like hormone (PTH1H) and is particularly abundant in brain and pancreas.

Description

Human PTH2R ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human PTH2R. 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

PTH2R (Genbank Accession No. NP_005039)

Applications

- cAMP dependent human PTH2R cell based assay
- cell based high-throughput screening of human PTH2R inhibitors

Functional Test

- this cell line has been tested positive for PTH2R 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 x 10⁶ cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250 µg/ml G418, 1 µg/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

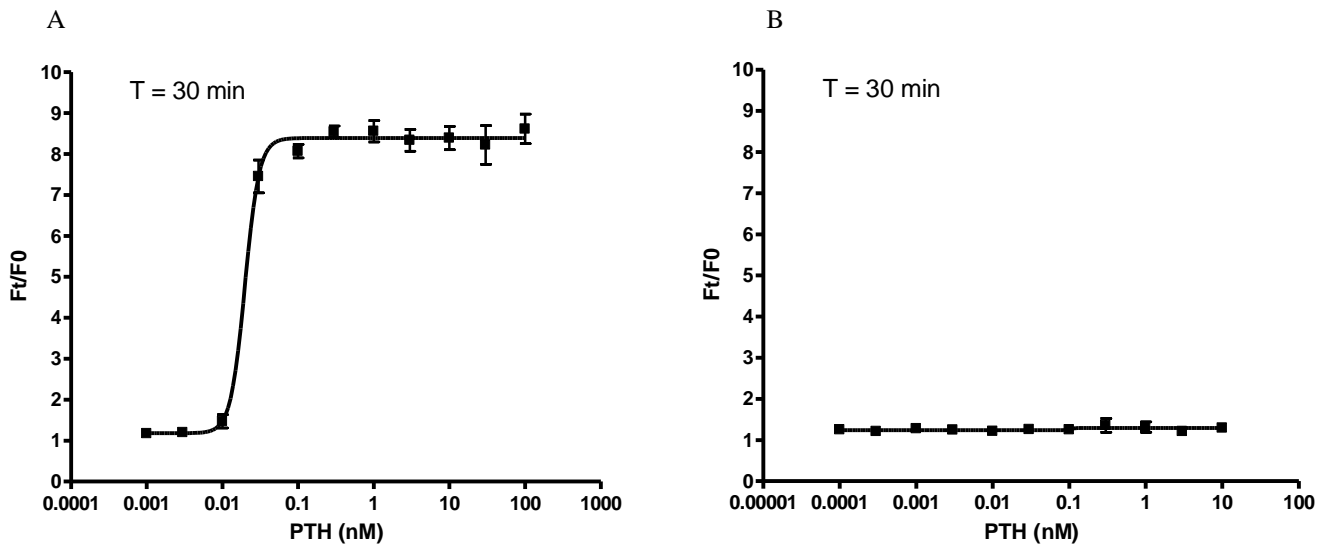


Figure 1. Response of ACTOne™ PTH2R cell line & parental cell line to PTH

ACTOne™ PTH2R cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20 µl culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 µl/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 PTH. Ratios of the two readings (F/F0) are plotted in the figure.

- A. Dose response curve of PTH in ACTOne™ PTH2R cell line. EC50 = 20 pM in the presence of PDE inhibitor Ro20-1724, and EC50 = 73.7 pM in the absence of Ro20-1724.**
- B. Parental cells do not respond to PTH.**

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