

## Parathyroid Hormone 1 Receptor (PTH1R) ACTOne™ Stable Cell Line

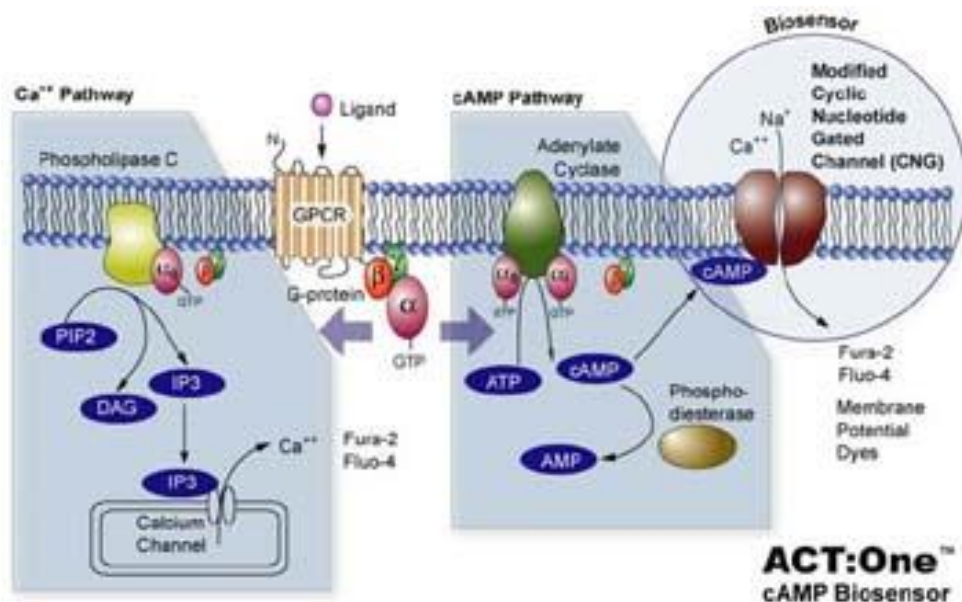
CATALOG NUMBER: CL-01-PTH1R

### Introduction

PTH1R a member of the secretin family of G protein-coupled receptors, regulated by parathyroid hormone (PTH) and for parathyroid hormone-like hormone (PTHrLH). The activity of this receptor is mediated by Gs G proteins which activate adenylyl cyclase. Besides, they also activate phosphatidylinositol-calcium second messenger system.

### Description

Human PTH1R ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human PTH1R. 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

PTH1R (Genbank Accession No. NP\_000307)

### Applications

- cAMP dependent human PTH1R cell based assay
- cell based high-throughput screening of human PTH1R inhibitors

### Functional Test

- this cell line has been tested positive for PTH1R 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

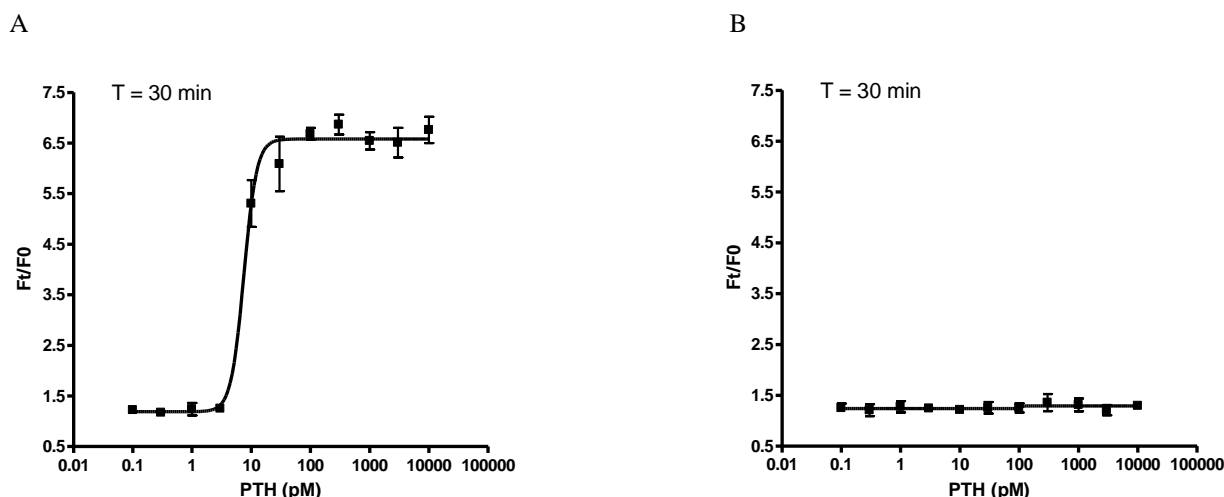
## Cell Culture Medium

- 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™ PTH1R cell line & parental cell line to PTH**

ACTOne™ PTH1R 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 PTH. Ratios of the two readings (F/F0) are plotted in the figure.

- Dose response curve of PTH in ACTOne™ PTH1R cell line. EC50 = 7.5 nM in the presence of PDE inhibitor Ro 20-1724.**
- Parental cells do not respond to PTH.**

## Notice to Purchaser

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