

Adenylate Cyclase Activating Polypeptide 1 Receptor (PAC1R) ACTOne™ Stable Cell Line

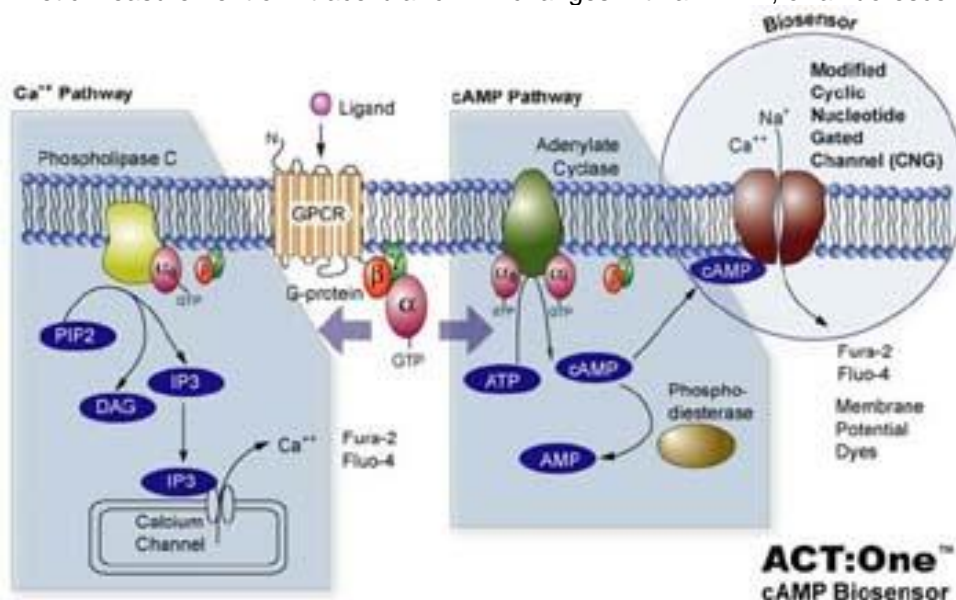
CATALOG NUMBER: CL-01-PAC1R

Introduction

PAC1R is a membrane-associated protein and shares significant homology with the members in the glucagon/secretin receptor family. This receptor mediates diverse biological actions of adenylate cyclase activating polypeptide 1 and is positively coupled to adenylate cyclase.

Description

Human PAC1R ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human PAC1R. 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

PAC1R (Genbank Accession No. P41586)

Applications

- cAMP dependent human PAC1R cell based assay
- cell based high-throughput screening of human PAC1R inhibitors

Functional Test

- this cell line has been tested positive for PAC1R 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.

Data Analysis

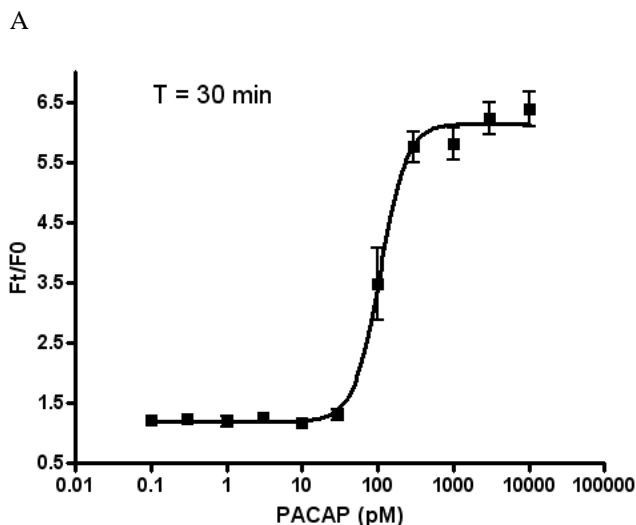


Figure 1. Response of ACTOne™ PAC1R cell line to PACAP

ACTOne™ PAC1R cells and the parental cells (Cat# CL-03-PC20) were plated overnight in 20 µl culture medium on a 384 well Biocoat plate. The next day, the 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 PACAP. Ratios of the two readings (F/F₀) are plotted in the figure.

- Dose response curve of PACAP in ACTOne™ PAC1R cell line. EC₅₀ = 107.5 pM in the absence of Ro20-1724.
- Parental cells do not respond to PACAP (data not shown).

Notice to Purchaser

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