Prostaglandin E Receptor 4 (EP4R) ACTOne™ Stable Cell Line

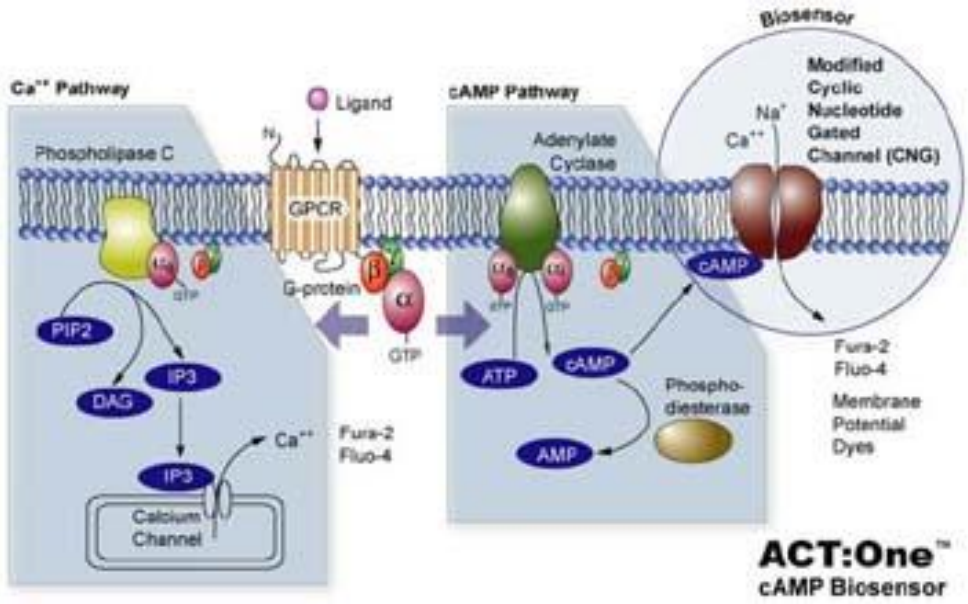
CATALOG NUMBER: CL-01-EP4R

Introduction

EP4R is a member of the G-protein coupled receptor family. This protein is one of four receptors identified for prostaglandin E2 (PGE2). This receptor can activate T-cell factor signaling. It has been shown to mediate PGE2 induced expression of early growth response 1 (EGR1), regulate the level and stability of cyclooxygenase-2 mRNA, and lead to the phosphorylation of glycogen synthase kinase-3. Knockout studies in mice suggest that this receptor may be involved in the neonatal adaptation of circulatory system, osteoporosis, as well as initiation of skin immune responses.

Description

Human EP4R ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human EP4R. 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

EP4R (Genbank Accession No. NP_000949)

Applications

- cAMP dependent human EP4R cell based assay
- cell based high-throughput screening of human EP4R inhibitors

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Functional Test

- this cell line has been tested positive for EP4R 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 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 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

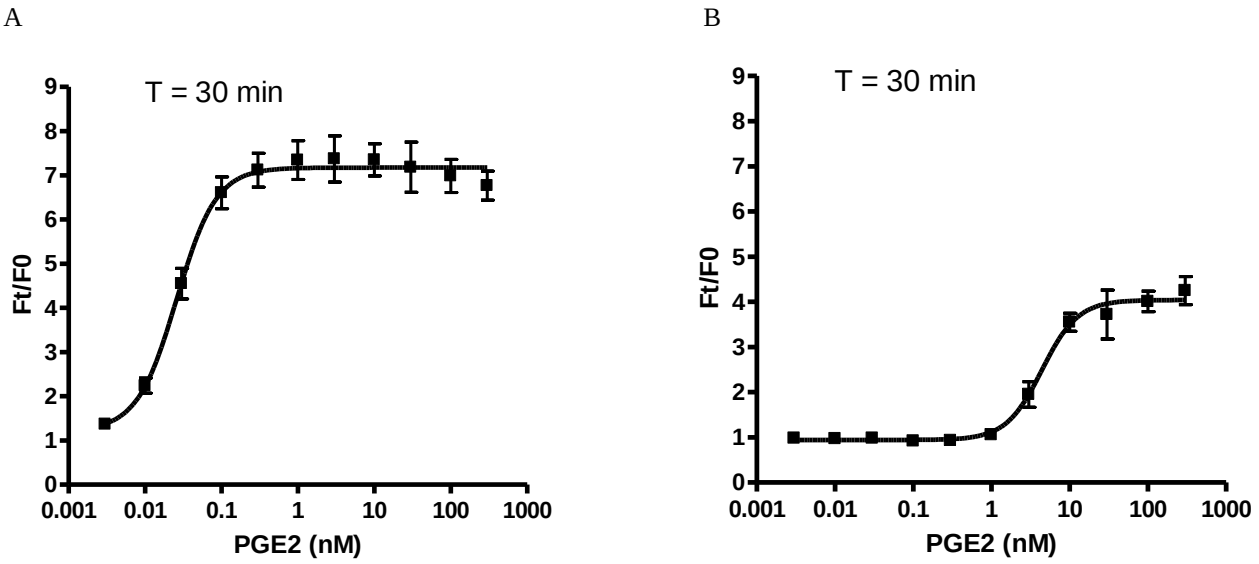

Figure 1. Response of ACT*One*™ EP4R cell line & parental cell line to PGE2.

ACTOne™ EP4R 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 glucagon. Ratios of the two readings (F/F0) are plotted in the figure.

A. Dose response curve of PGE2 in ACT*One*™ EP4R cell line. EC50 = 26.2 nM in the presence of PDE inhibitor Ro 20-1724, and EC50 = 121 pM in the absence of Ro20-1724 (data shown).

B. Parental cells do not respond to PGE2 when the concentrations are less than 1 nM (in presence of PDE inhibitor). In the absence of PDE inhibitor, parental cells do not respond to PGE2 when the concentrations are less than 10 nM (data not shown).

Notice to Purchaser

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