Prostaglandin E2 Receptor (PTGER2) ACTOne™ Stable Cell Line

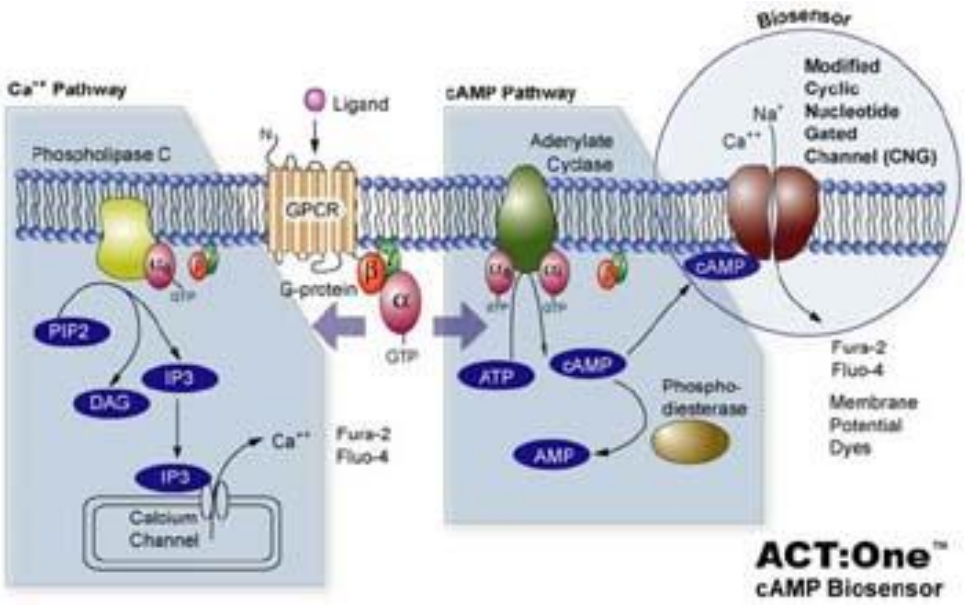
CATALOG NUMBER: CL-01-PTGER2

Introduction

PTGER2 is a G-protein coupled receptor for prostaglandin E2, a metabolite of arachidonic acid which has different biologic activities in a wide range of tissues. The activity of this receptor is mediated by G(s) proteins that stimulate adenylate cyclase. The subsequent raise in intracellular cAMP is responsible for the relaxing effect of this receptor on smooth muscle. Mutations in PTGER2 gene are associated with aspirin-induced susceptibility to asthma.

Description

Human PTGER2 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human PTGER2. 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

PTGER2 (Genbank Accession No. XP_007322.1)

Applications

- cAMP dependent human PTGER2 cell based assay
- cell based high-throughput screening of human PTGER2 inhibitors

Functional Test

- this cell line has been tested positive for PTGER2 specific response

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- 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

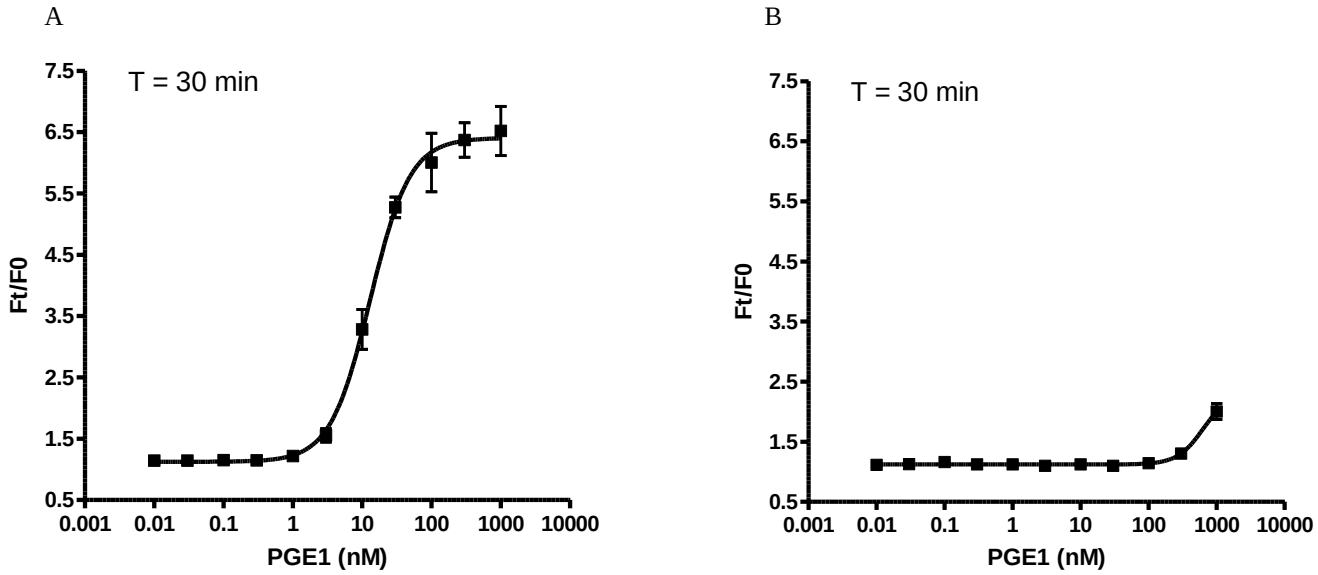

Figure 1. Response of ACT*One*™ PTGER2 cell line & parental cell line to PGE1.

ACTOne™ PTGER2 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 PGE1. Ratios of the two readings (F/F0) are plotted in the figure.

A. Dose response curve of PGE1 in ACT*One*™ PTGER2 cell line. EC50 = 13.0 nM in the absence of PDE inhibitor Ro 20-1724.

B. Parental cells do not respond to PGE1.

Notice to Purchaser

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