

Cannabinoid Receptor 1 (CB1) ACTOne™ Stable Cell Line

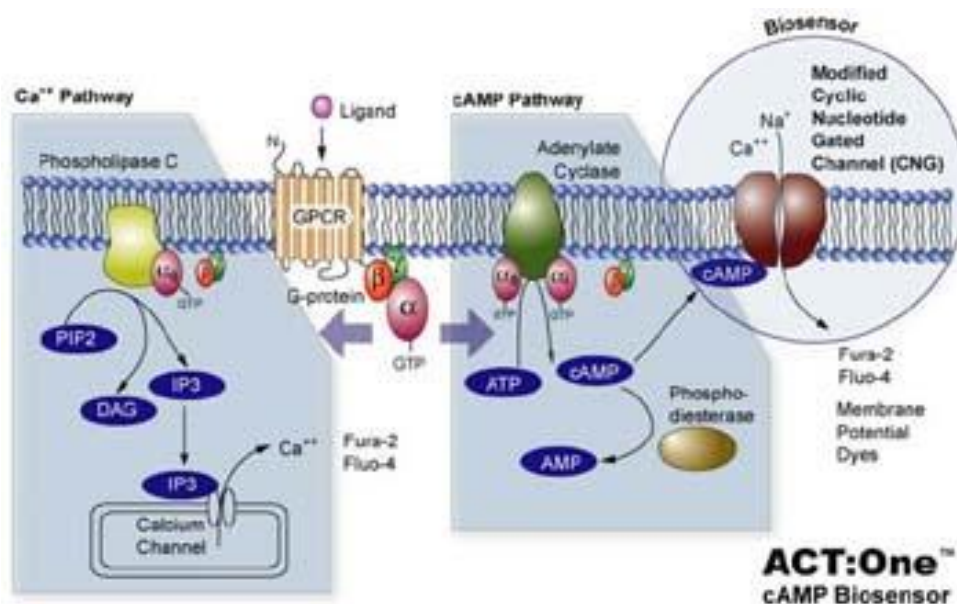
CATALOG NUMBER: CL-11-CB1

Introduction

The CB1 receptor, also known as the central cannabinoid receptor, is a member of the cannabinoid receptor group of G protein-coupled receptors that also includes CB2 and GPR55. Upon activation, CB1 receptor exhibits its effects mainly through activation of Gi, which decreases intracellular cAMP concentration by inhibiting its production enzyme, adenylate cyclase, and increases mitogen-activated protein kinase (MAP kinase) concentration.

Description

Human CB1 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human CB1. 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

CB1 (Genbank Accession No. P21554)

Applications

- cAMP dependent assay for Gi-coupled human CB1
- cell based high-throughput screening of human CB1 inhibitors

Functional Test

- this cell line has been tested positive for CB1 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 (1×10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250 μ g/ml G418, 1 μ 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°C , preferably in liquid nitrogen vapor, until ready for use.

Data Analysis

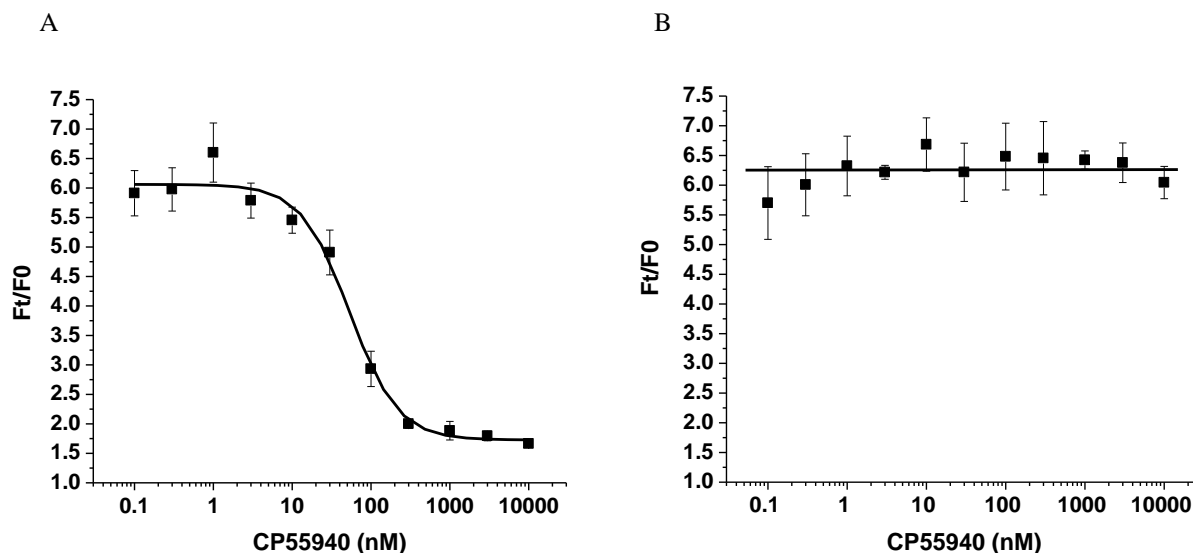


Figure 1. Response of ACTOne™ CB1 cell line & parental cell line to CP-55940.

ACTOne™ CB1 cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20 μ l culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 μ 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 CP-55940. Ratios of the two readings (F/F0) are plotted in the figure.

- Dose response curve of CP-55940 in ACTOne™ CB1 cell line. EC50 = 53nM in the presence of PDE inhibitor Ro 20-1724 and β -adrenoceptor agonist isoproterenol.**
- Parental cells do not respond to CP-55940.**

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