

## Cannabinoid Receptor 2 (CB2) ACTOne™ Stable Cell Line

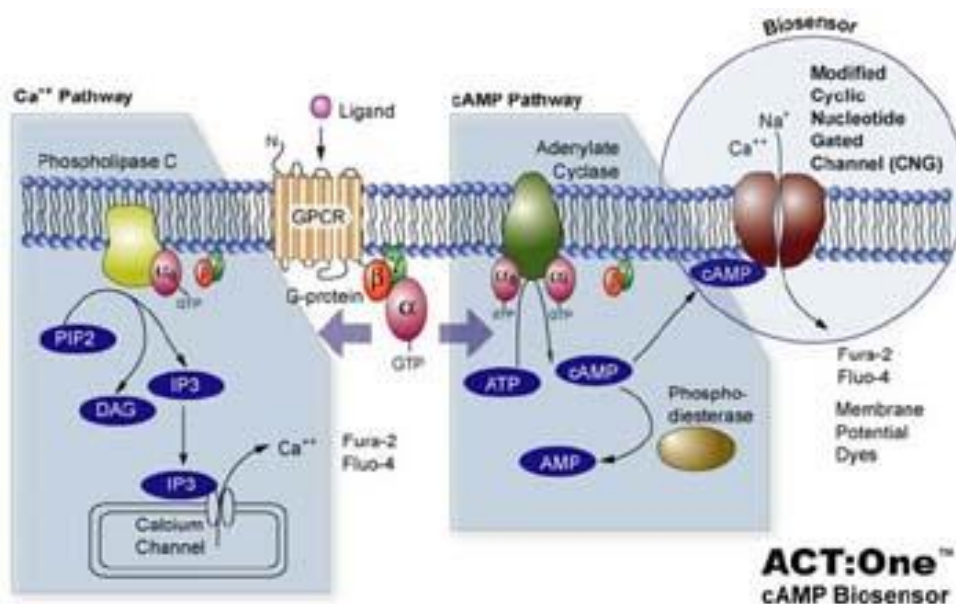
CATALOG NUMBER: CL-11-CB2

### Introduction

The CB2 is a member of the G protein-coupled receptor family 1. It is closely related to the cannabinoid receptor type 1, which is largely responsible for the efficacy of endocannabinoid-mediated presynaptic-inhibition, the psychoactive properties of tetrahydrocannabinol, the active agent in marijuana, and other phytocannabinoids (natural cannabinoids). The principal endogenous ligand for the CB2 receptor is 2-arachidonoylglycerol (2-AG).

### Description

Human CB2 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human CB2. 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

CB2 (Genbank Accession No. NP\_001832)

### Applications

- cAMP dependent assay for Gi-coupled human CB2
- cell based high-throughput screening of human CB2 inhibitors

### Functional Test

- this cell line has been tested positive for CB2 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

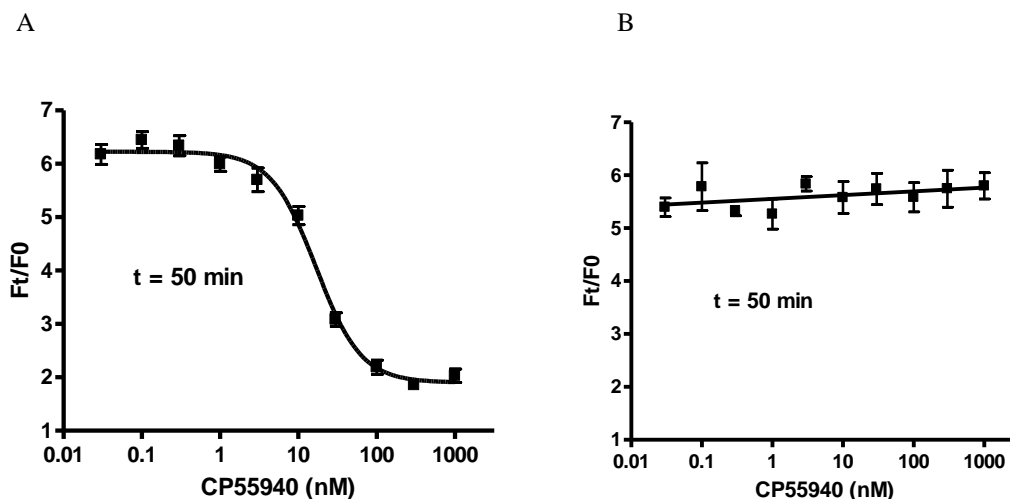
## Cell Culture Medium

- 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™ CB2 cell line & parental cell line to CP-55940.**

ACTOne™ CB2 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 CP-55940. Ratios of the two readings (Ft/F0) are plotted in the figure.

- Dose response curve of CP-55940 in ACTOne™ CB2 cell line. EC50 = 16.7nM in the presence of 25  $\mu$ M of PDE inhibitor Ro 20-1724 and 250 nM of adenosine A2b receptor agonist NECA.**
- Parental cells do not respond to CP-55940.**

## Notice to Purchaser

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