Opioid Receptor, Mu 1 (OPRM1) ACTOne™ Stable Cell Line

CATALOG NUMBER: CL-11-OPRM1

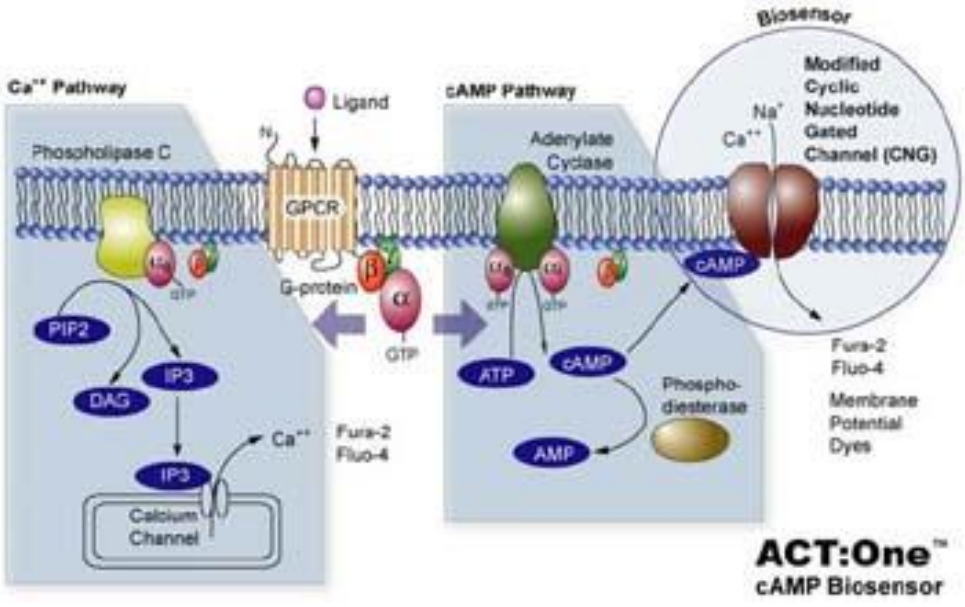
Introduction

Three variants (mu1, mu2, mu3) of the mu opioid receptor exist due to the alternative splicing. The mu opioid receptor is a member of the opioid family of G-protein-coupled receptors that also includes kappa, delta and NOP receptors. The mu opioid receptor is the principal target of endogenous opioids such as beta-endorphin and endomorphin and natural or synthetic opioids including morphine, heroin, DAMGO, fentanyl, etorphine, buprenorphin and methadone.

Mu Opioid receptors are distributed throughout the neuraxis (neocortex, thalamus, nucleus accumbens, hippocampus, amygdala) and in the peripheral nervous system (myenteric neurons and vas deferens). mu Opioid receptors have been implicated in respiration, cardiovascular functions, feeding, learning and memory, intestinal transit, locomotor activity, thermoregulation, hormone secretion and immune functions.

Description

Human OPRM1 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human OPRM1. 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

OPRM1 (Genbank Accession No. AAS83107)

Applications

- cAMP dependent assay for Gi-coupled human OPRM1

Tel: 1-800-919-0755 • Fax: 1-240-683-5852 • info@eEnzyme.com • www.eEnzyme.com

- cell based high-throughput screening of human OPRM1 inhibitors

Functional Test

- this cell line has been tested positive for OPRM1 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

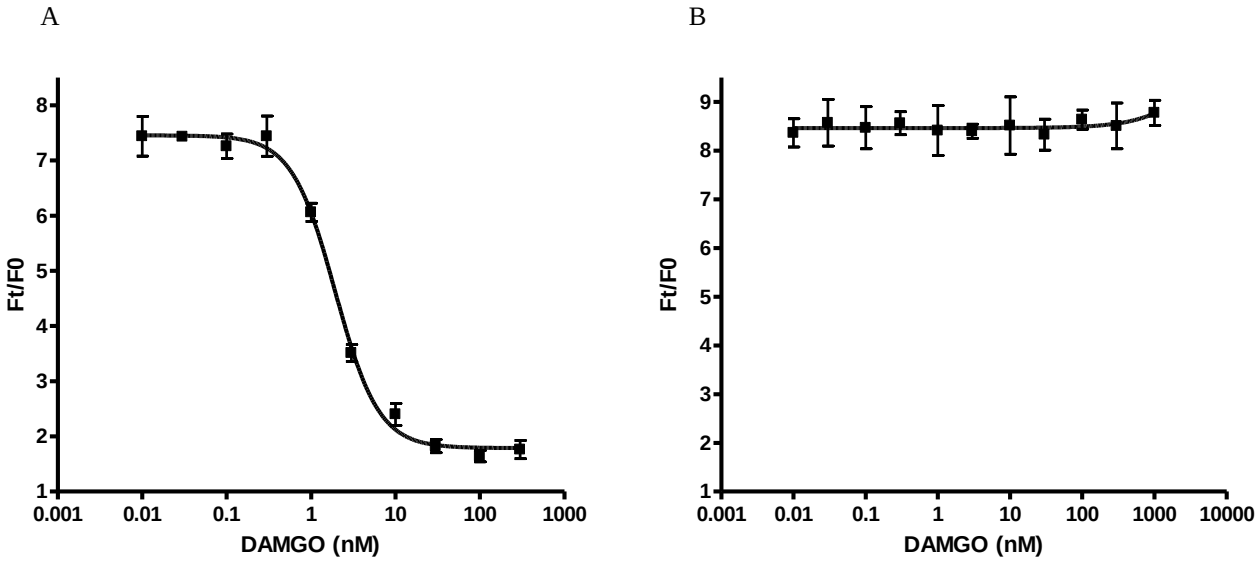


Figure 1. Response of ACT*One*™ OPRM1 cell line & parental cell line to DAMGO.

ACTOne™ OPRM1 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 DAMGO. Ratios of the two readings (F/F0) are plotted in the figure.

A. Dose response curve of DAMGO in ACT*One*™ OPRM1 cell line. EC50 = 1.9 nM in the presence of PDE inhibitor Ro 20-1724 and β-adrenoceptor agonist isoproterenol.

B. Parental cells do not respond to DAMGO.

Tel: 1-800-919-0755 • Fax: 1-240-683-5852 • info@eEnzyme.com • www.eEnzyme.com

Notice to Purchaser

This cell line is sold with a use license. It may not be transferred to third parties, resold, modified for resale without written approval of eEnzyme LLC. Refer to the license agreements for details on the usage restrictions.