Beta-2 Adrenergic Receptor (ADRB2) ACTOne™ Stable Cell Line

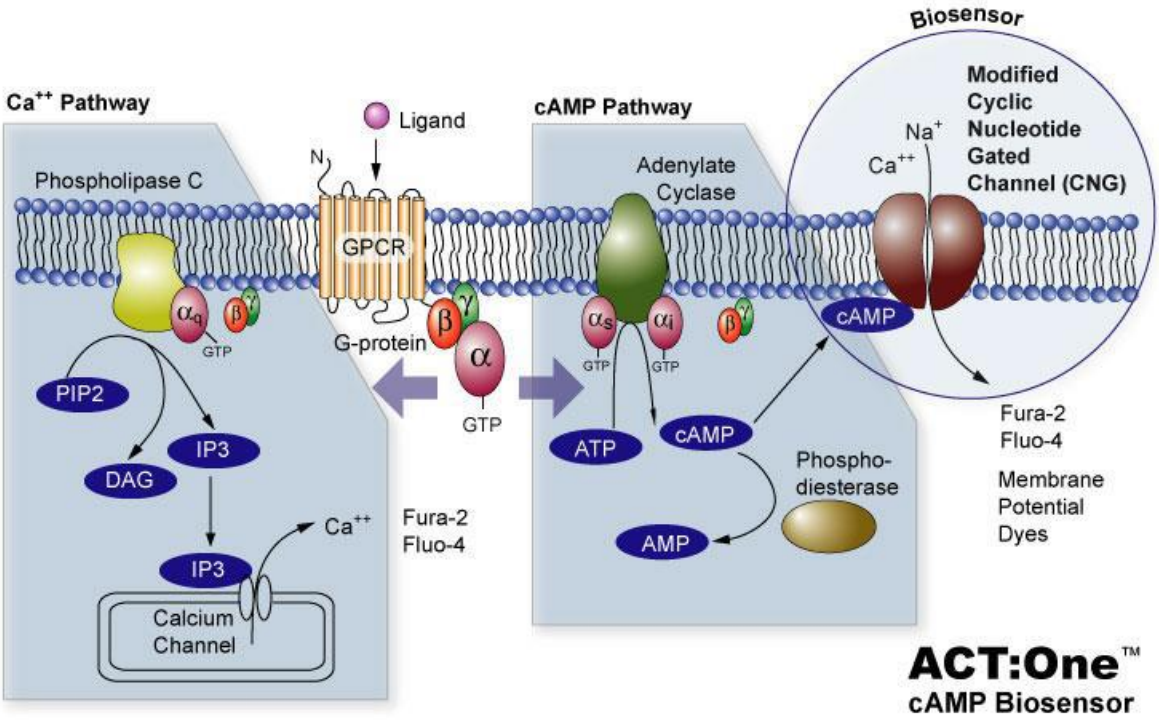
CATALOG NUMBER: CL-01-ADRB2

Introduction

ADRB2 is a member of the G protein-coupled receptor superfamily. This receptor is directly associated with one of its ultimate effectors, the class C L-type calcium channel Ca(V)1.2. This receptor-channel complex also contains a G protein, an adenylyl cyclase, cAMP-dependent kinase, and the counterbalancing phosphatase, PP2A. The assembly of the signaling complex provides a mechanism that ensures specific and rapid signaling by this G protein-coupled receptor.

Description

Human ADRB2 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human ADRB2. 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

ADRB2 (Genbank Accession No. AAA88017.1)

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

Applications

- cAMP dependent human ADRB2 cell based assay
- cell based high-throughput screening of human ADRB2 inhibitors

Functional Test

- this cell line has been tested positive for ADRB2 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.

Assay materials not included

10X Elite™ Membrane Potential Assay Kit	eEnzyme CA-M165
Biocoat Poly-D-Lysine coated 384-well black/clear plate	BD 354663
Phosphodiesterase (PDE) inhibitor Ro 20-1724 (50mM stock in DMSO, store at -20°C)	Sigma B8279
Dulbecco's Phosphate Buffered Saline (DPBS)	Sigma D8537
Isoproterenol (10 mM stock in dH2O)	Sigma I6504

Cell culture materials not included

DMEM, high glucose, with glutamine	Biosource International P104G-000
Fetal bovine serum	Invitrogen 26140-079
Trypsin-EDTA solution (10x)	Sigma T4174
G418 sulfate	Cellgro 61-234-RG
Puromycin	Clontech 8052-2
Blasticidin S HCl	Invitrogen R210-01

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

Data Analysis

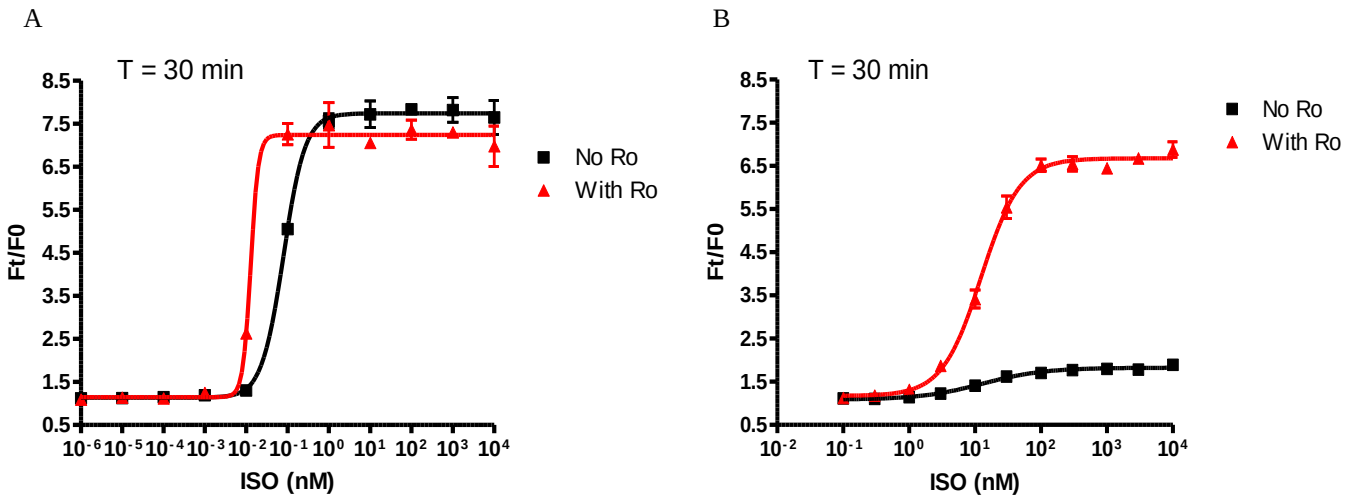


Figure 1. Response of ACT*One*™ ADRB2 cell line & parental cell line to Isoproterenol.
ACTOne™ ADRB2 cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20 µl culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 µl/well of 1x Dye-loading solution (membrane potential dye kit, Cat# CA-M165). After 2 hour of incubation at room temperature, two readings were obtained prior to and 30 min after the addition of Isoproterenol. Ratios of the two readings (F/F0) are plotted in the figure..

A. Dose response curve of Isoproterenol in ACT*One*™ ADRB2 cell line. EC50 = 12.8 pM in the presence of 25 µM of PDE inhibitor Ro20-1724 and EC50 = 79.9 pM in the absence of PDE inhibitor Ro20-1724.

B. EC50 of Isoproterenol is 12.39 nM in the parental cells in the presence of 25 µM of PDE inhibitor Ro20-1724. In the absence of PDE inhibitor, there is not much response to Isoproterenol in the parental cells.

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.