

Beta-1 Adrenergic Receptor (ADRB1) ACTOne™ Stable Cell Line

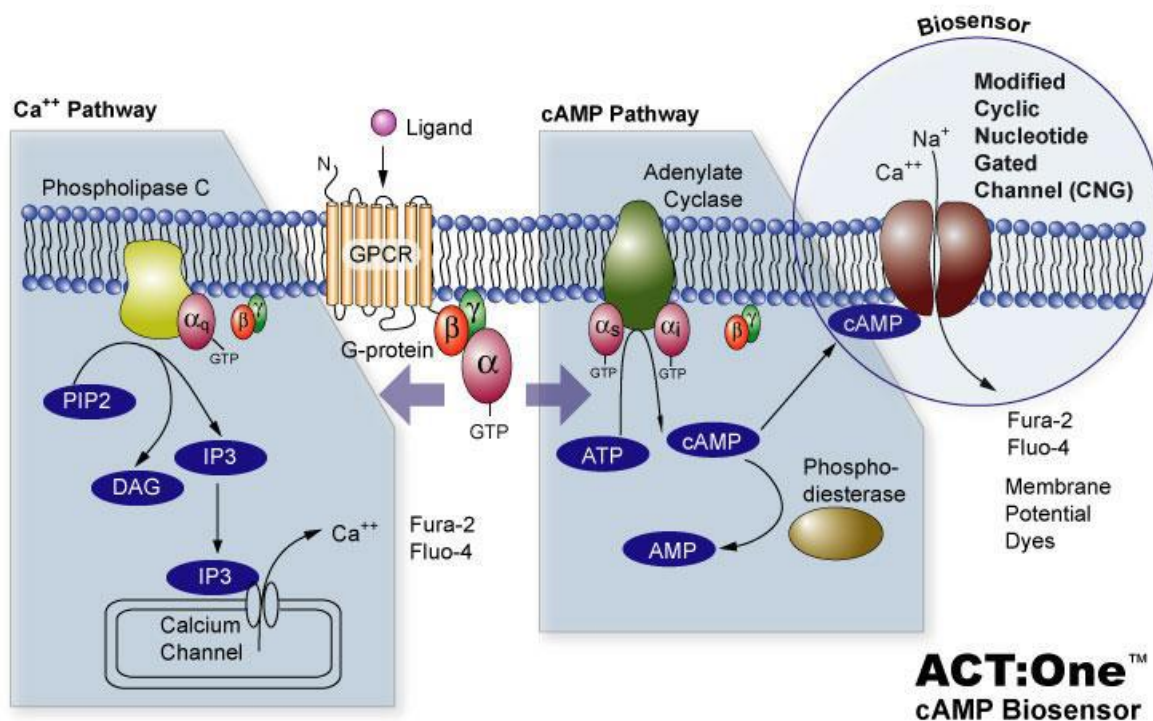
CATALOG NUMBER: CL-01-ADRB1

Introduction

ADRB1 is a G-protein coupled receptor associated with the Gs heterotrimeric G-protein and is expressed predominantly in cardiac tissue.

Description

Human ADRB1 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human ADRB1. 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

ADRB1 (Genbank Accession No. NP_000675.1)

Applications

- cAMP dependent assay for Gs-coupled human ADRB1 receptor
- cell based high-throughput screening of human ADRB1 inhibitors

Functional Test

- this cell line has been tested positive for ADRB1 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

Tested and found to be free of mycoplasma contamination.

Content

- Stable cells: 1 mL (1 x 10⁶ 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
Norepinephrine (0.1 M stock in 0.5 M HCl)	Sigma A7257
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



Data Analysis

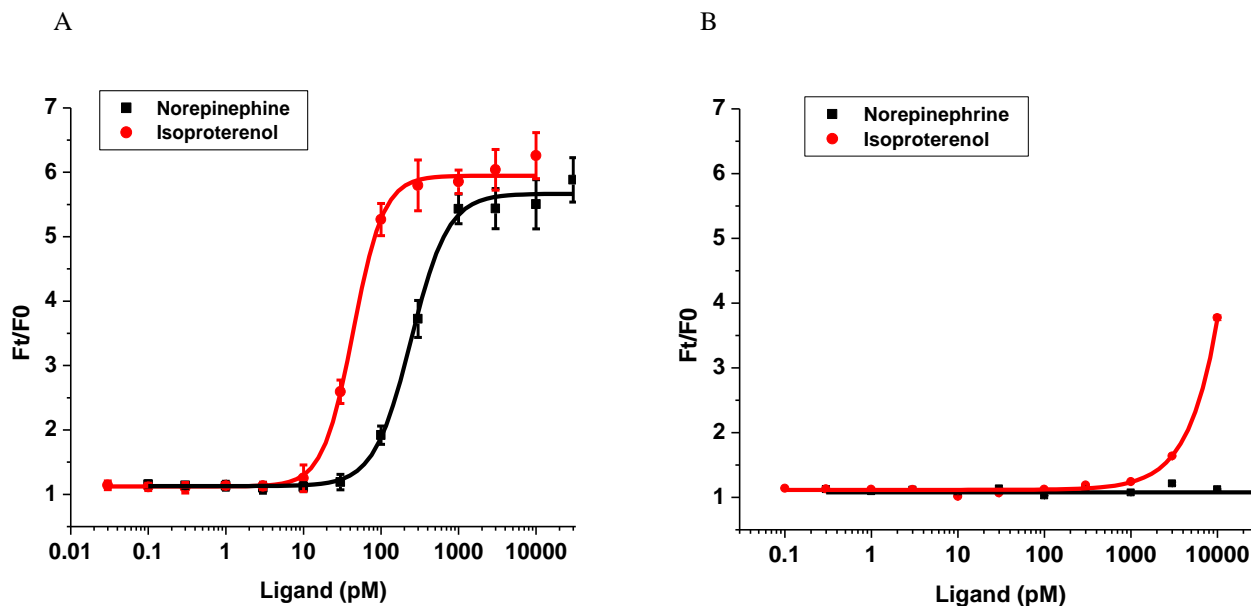


Figure 1. Response of ACTOne™ ADRB1 cell line & parental cell line to Norepinephrine and Isoproterenol.

ACTOne™ ADRB1 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 Isoproterenol. Ratios of the two readings (F/F0) are plotted in the figure.

- A. Dose response curve of Norepinephrine or Isoproterenol in ACTOne ADRB1 cell line. With Norepinephrine, EC50 = 243 pM in the presence of 25 µM of PDE inhibitor Ro20-1724; With Isoproterenol, EC50 = 43.8 pM in the presence of 25 µM of PDE inhibitor Ro20-1724
- B. Parental cells do not respond to Norepinephrine. The Parental cells do not respond to Isoproterenol when it is lower than 1 nM.

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.