Adenosine A3 Receptor (ADORA3) ACTOne™ Stable Cell Line

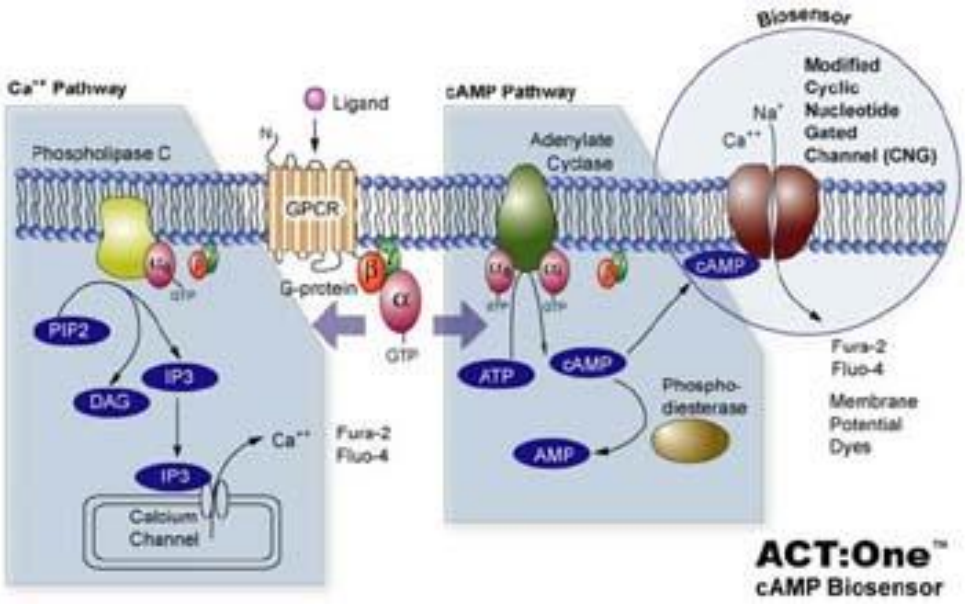
CATALOG NUMBER: CL-11-ADORA3

Introduction

Adenosine A3 receptors (ADORA3) are G protein-coupled receptors that couple to Gi/Gq and are involved in a variety of intracellular signaling pathways and physiological functions. It mediates a sustained cardioprotective function during cardiac ischemia, it is involved in the inhibition of neutrophil degranulation in neutrophil-mediated tissue injury, it has been implicated in both neuroprotective and neurodegenerative effects, and it may also mediate both cell proliferation and cell death. Recent publications demonstrate that adenosine A3 receptor antagonists (SSR161421) could have therapeutic potential in bronchial asthma.

Description

Human ADORA3 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human ADORA3. 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

ADORA3 (Genbank Accession No. P33765)

Applications

- cAMP dependent assay for Gi-coupled human ADORA3
- cell based high-throughput screening of human ADORA3 inhibitors

Functional Test

- this cell line has been tested positive for ADORA3 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

A B

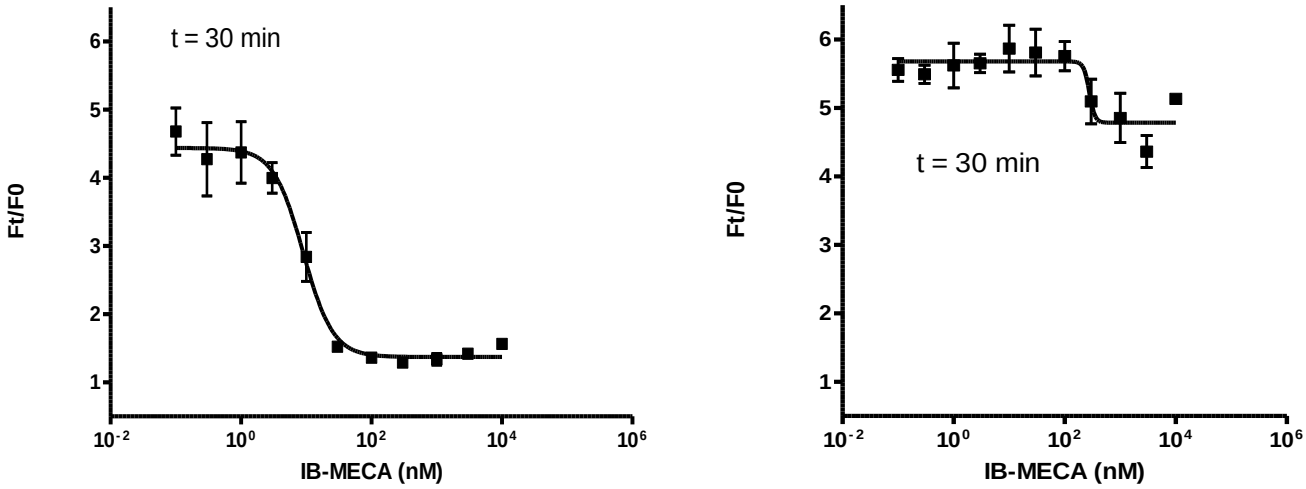


Figure 1. Response of ACT*One*™ ADORA3 cell line & parental cell line to IB-MECA.

ACTOne™ ADORA3 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 IB-MECA. Ratios of the two readings (F/F0) are plotted in the figure.

A. Dose response curve of IB-MECA in ACT*One*™ ADORA3 cell line. EC50 = 9nM in the presence of PDE inhibitor Ro 20-1724 and β–adrenoceptor agonist isoproterenol.

B. Parental cells do not respond to IB-MECA.

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