

Adenosine A2A Receptor (ADORA2A) ACTOne™ Stable Cell Line

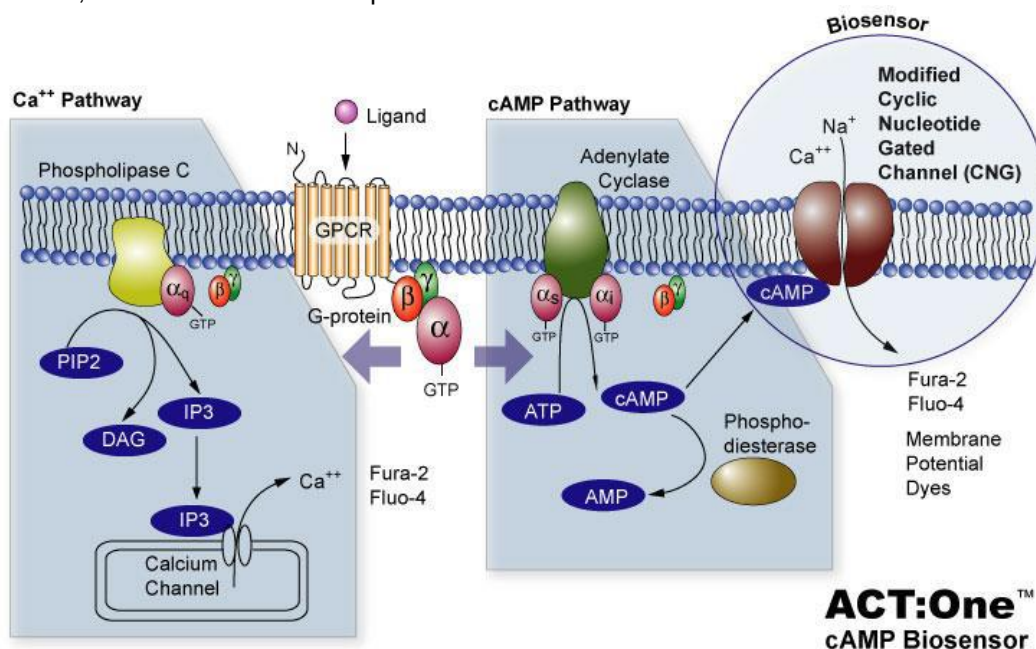
CATALOG NUMBER: CL-01-ADORA2A

Introduction

ADORA2A is a member of the guanine nucleotide-binding protein (G protein)-coupled receptor (GPCR) superfamily, which is subdivided into classes and subtypes. The receptors are seven-pass transmembrane proteins that respond to extracellular cues and activate intracellular signal transduction pathways. This protein, an adenosine receptor of A2A subtype, uses adenosine as the preferred endogenous agonist and preferentially interacts with the G(s) protein to increase intracellular cAMP levels. It plays an important role in many biological functions, such as cardiac rhythm and circulation, cerebral and renal blood flow, immune function, pain regulation, and sleep. It has been implicated in pathophysiological conditions such as inflammatory diseases and neurodegenerative disorders.

Description

Human ADORA2A ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human ADORA2A. 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 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

ADORA2A (Genbank Accession No. NP_000666)

Applications

- cAMP dependent cell based assay for G_s-coupled human ADORA2A
- cell based high-throughput screening of human ADORA2A activators/inhibitors

Functional Test

- this cell line has been tested positive for ADORA2A specific response
- surviving rate: More than 1 million/vial on the second day after thawing
- the receptor specific activity is stable for 10 weeks continuous passage

Mycoplasma Contamination Test

The cells have been tested and found to be free of *mycoplasma* contamination.

Content

- ADORA2A Stable cells: 1 mL (>1 x 10⁶ cells/mL in 80% DMEM, 10% FBS, 10% DMSO)

Growth Property: 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

Data Analysis

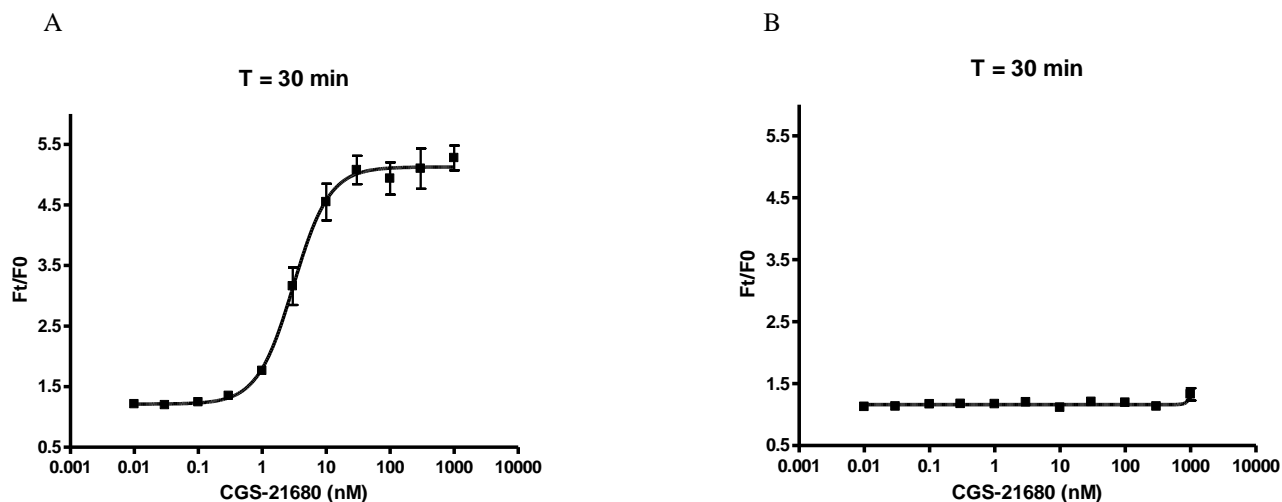


Figure 1. Response of ACTOne™ ADORA2A cell line & parental cell line to CGS-21680.

ACTOne™ ADORA2A 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 CGS-21680. Ratios of the two readings (F/F₀) are plotted in the figure.

- A. Dose response curve of CGS-21680 in ACTOne™ ADORA2A cell line. EC₅₀ = 3.1 nM**
- B. Parental cells do not respond to CGS-21680.**

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