

## Adenosine A1 Receptor (ADORA1) ACTOne™ Stable Cell Line

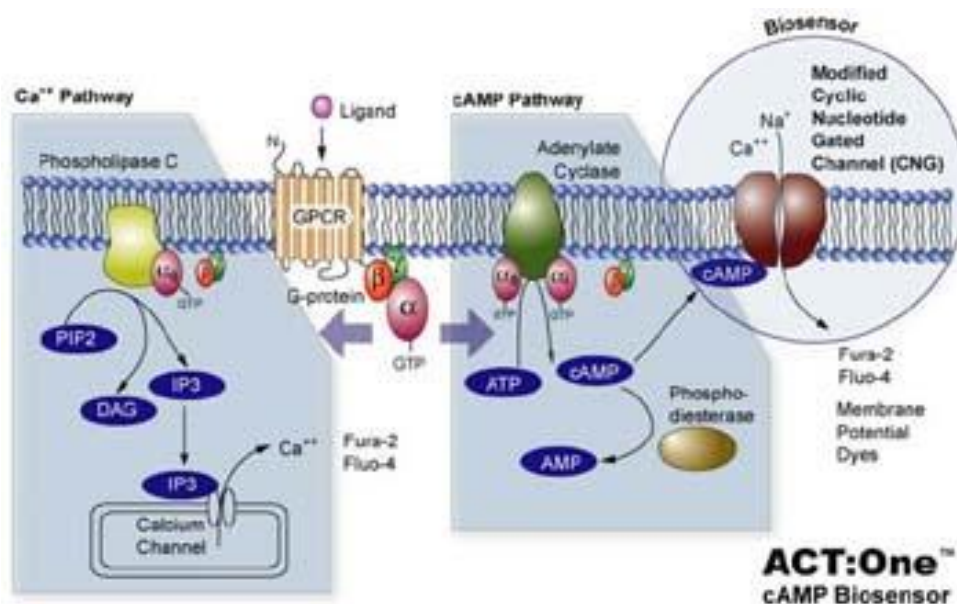
CATALOG NUMBER: CL-11-ADORA1

### Introduction

The adenosine A1 receptor (ADORA1) is one member of the adenosine receptor group of G protein-coupled receptors with adenosine as endogenous ligand. Adenosine A1 receptors are implicated in sleep promotion by inhibiting wake-promoting cholinergic neurons in the basal forebrain. Adenosine A1 receptors are also present in smooth muscle throughout the vascular system.

### Description

Human ADORA1 ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human ADORA1. 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

ADORA1 (Genbank Accession No. NP\_000665)

### Applications

- cAMP dependent assay for Gi-coupled human ADORA1
- cell based high-throughput screening of human ADORA1 inhibitors

### Functional Test

- this cell line has been tested positive for ADORA1 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 ( $1 \times 10^6$  cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

### Growth Properties

Adherent

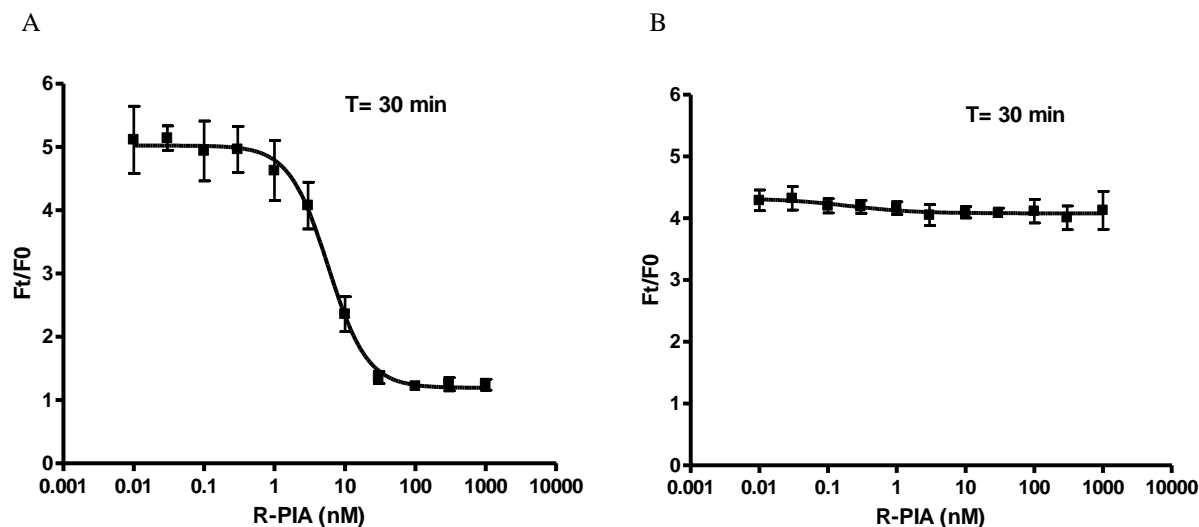
### Cell Culture Medium

- Growth medium: DMEM-10% FBS supplemented with 250  $\mu$ g/ml G418, 1  $\mu$ g/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  $-130^{\circ}\text{C}$ , preferably in liquid nitrogen vapor, until ready for use.

### Data Analysis



**Figure 1. Response of ACTOne™ ADORA1 cell line & parental cell line to R-PIA.**

ACTOne™ ADORA1 cells and parental cells (Cat# CL-03-PC20) were plated overnight in 20  $\mu$ l culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20  $\mu$ l/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 R-PIA. Ratios of the two readings (Ft/F0) are plotted in the figure.

- Dose response curve of R-PIA in ACTOne™ ADORA1 cell line. EC50 = 5.8 nM in the presence of PDE inhibitor Ro 20-1724 and  $\beta$ -adrenoceptor agonist isoproterenol.**
- Parental cells do not respond to R-PIA.**

### Notice to Purchaser

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