

## Adenosine A2b Receptor (ADORA2B) ACTOne™ Stable Cell Line

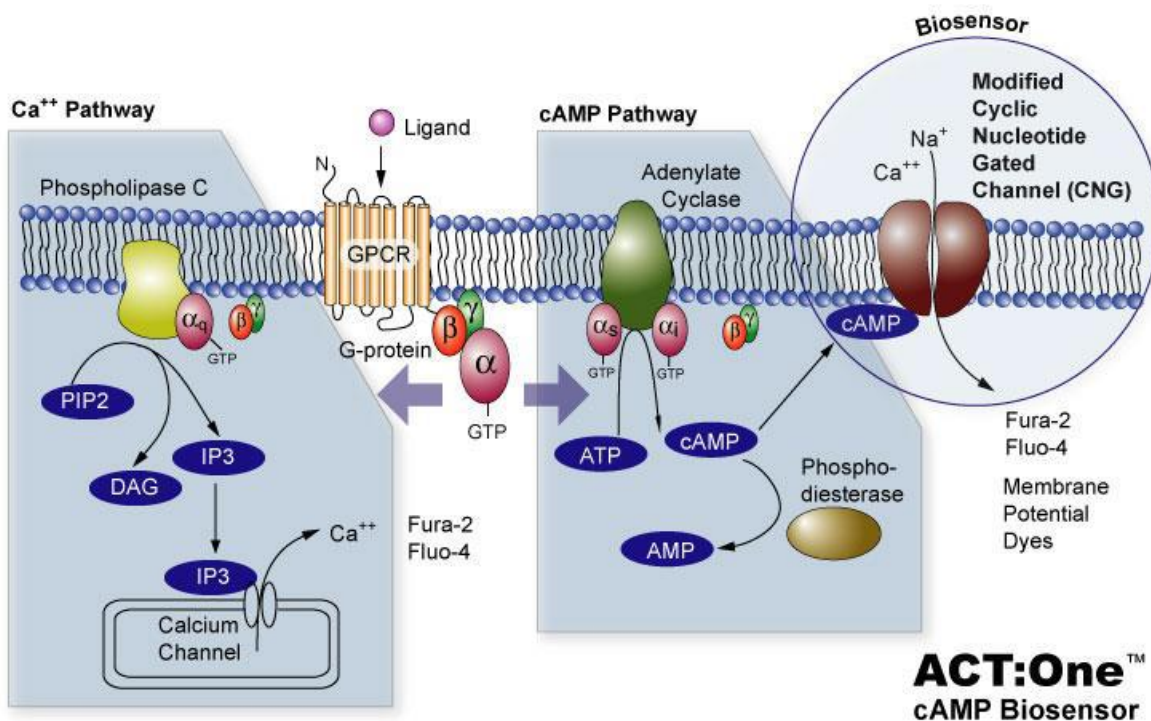
CATALOG NUMBER: CL-01-ADORA2B

### Introduction

ADORA2B is a member of the G protein-coupled receptor superfamily. This integral membrane protein stimulates adenylate cyclase activity in the presence of adenosine. This protein also interacts with netrin-1, which is involved in axon elongation

### Description

Human ADORA2B ACTOne™ is a HEK-293 CNG cell line that expresses recombinant human ADORA2B. 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 FDSS, 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

ADORA2B (Genbank Accession No. NP\_000667.1)

### Applications

- cAMP dependent human ADORA2B receptor cell based assay
- cell based high-throughput screening of human ADORA2B receptor agonists/antagonists

### Functional Tests

- this cell line has been tested positive for ADORA2B receptor specific response
- surviving rate: more than 2 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 have been tested and found to be free of mycoplasma contamination.

## Content

- Stable cells: 1 mL ( $2 \times 10^6$  cells/mL in DMEM, 20% FBS, 10% DMSO)

## Growth Properties

Adherent

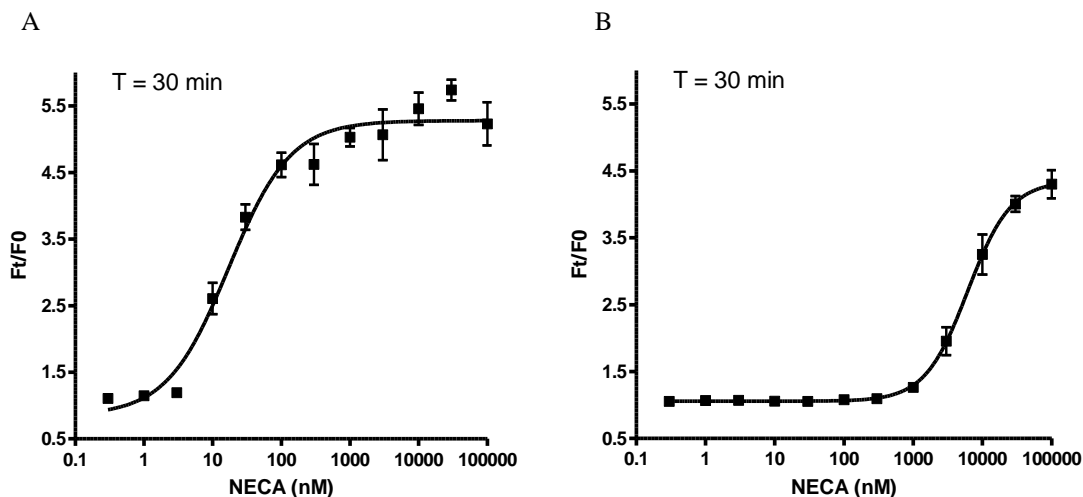
## Cell Culture Medium

- 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 Example



**Figure 1. Response of ACTOne™ ADORA2B receptor cell line & parental cell line to NECA.**

ACTOne™ ADORA2B receptor 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 NECA. Ratios of the two readings (F/F0) are plotted in the figure.

- Dose response curve of NECA in ACTOne™ ADORA2B cell line. EC50 = 17.08 nM in the absence of PDE inhibitor Ro 20-1724.**
- Dose response curve of NECA in the parental cell line. EC50 = 6.1  $\mu$ M in the absence of PDE inhibitor Ro20-1724.**

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