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Adenosine A2B Receptor (ADORA2B) ACTOne™ Stable Cell Line

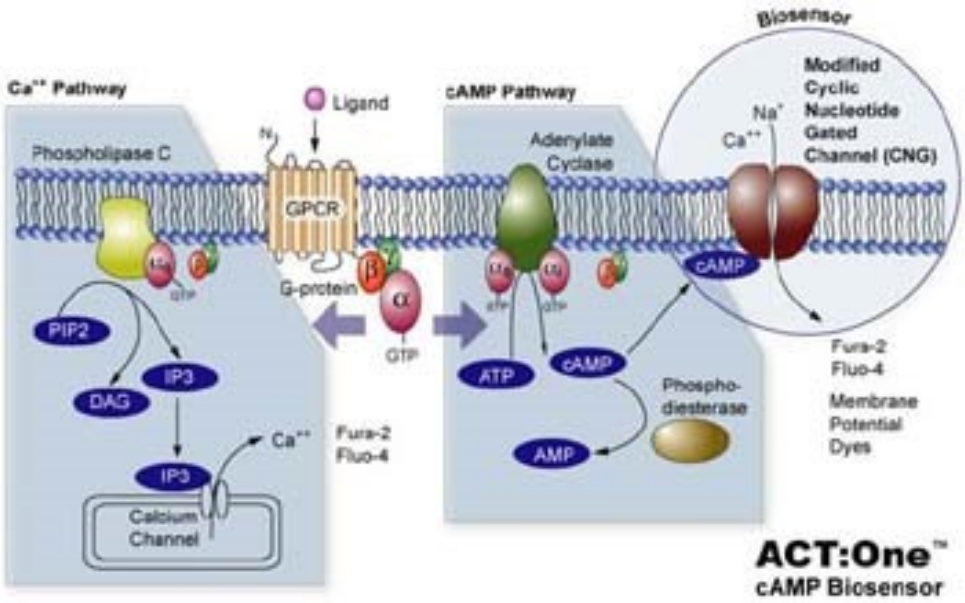
CATALOG NUMBER: CL-01-ADORA2B

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 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 Test

- this cell line has been tested positive for ADORA2B 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 (**for ADORA2B receptor stable cells**): 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:

Elite™ Membrane Potential Dye Kit	EENZYME Cat# 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
NECA [5'-(N-ethylcarboxamido) adenosine]	Sigma E2387

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

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Data Example

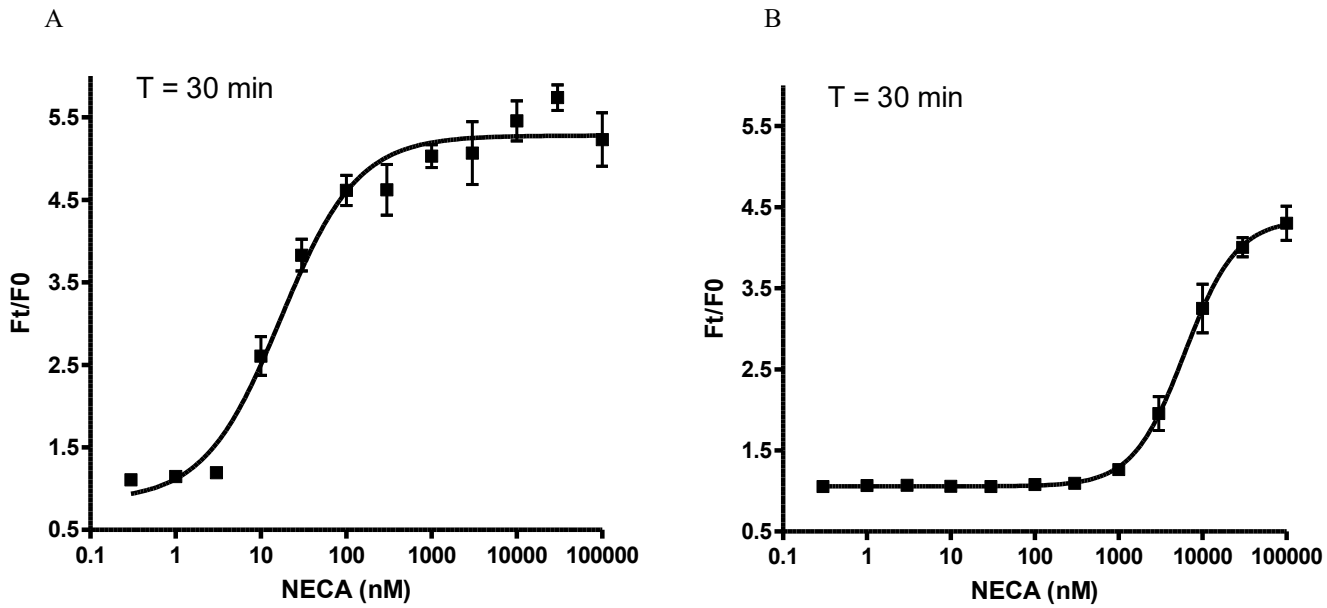

Figure 1. Response of ACT*One*™ ADORA2B cell line & parental cell line to NECA.

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

A. Dose response curve of NECA in ACT*One*™ ADORA2B cell line. EC50 = 17.08 nM.

B. Dose response curve of NECA in the parental cell line. EC50 = 6.1 µM in the absence of PDE inhibitor Ro20-1724.

Notice to Purchaser

1.This cell line is to be used for research purposes only. It may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without written approval of eEnzyme LLC.
2. Refer to the license agreement for details on the usage restrictions.