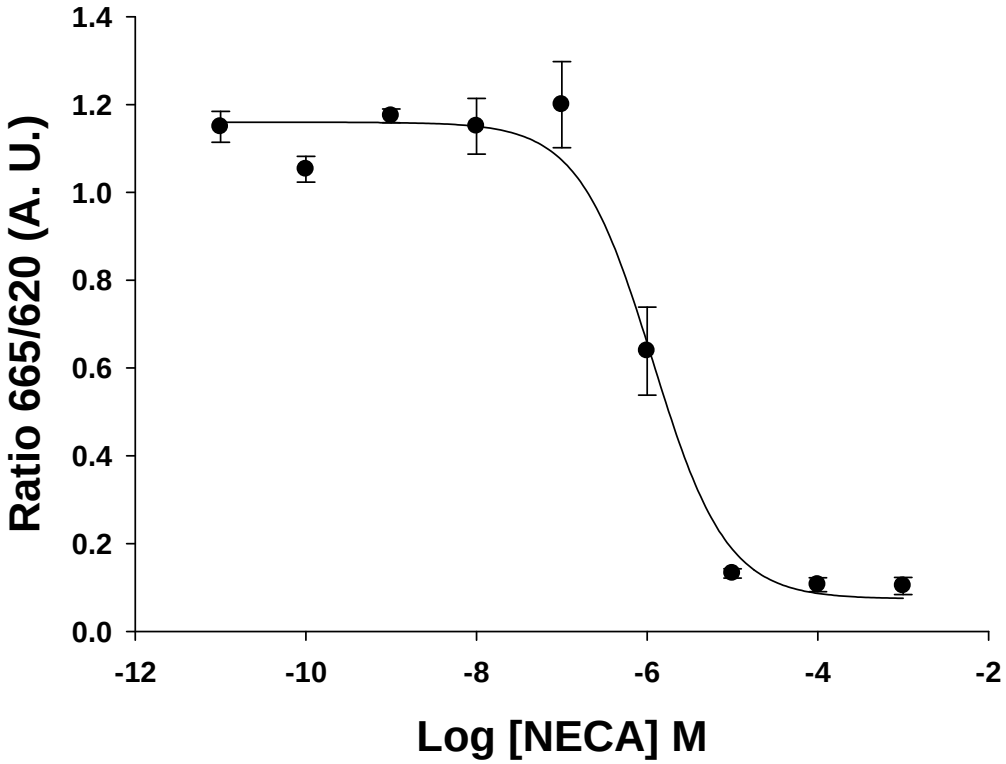
REF: P30192

# *HiTSeeker CELL LINES (LABEL-FREE GPCRS)*

## - ADENOSINE RECEPTOR A2B (ADORA2B) CELL LINE-




**Product name:** ADORA2B /HEK293 cell line

**$Ec_{50}$ NECA:** $1.16x10^{-6}$M

**Z´:** 0.84+/- 0.02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# - ADENOSINE RECEPTOR A2B (ADORA2B) CELL LINE -

| | |
|---|---|
| **Product Name:** | ADORA2B/HEK293 |
| **Official Full Name:** | Adenosine $A_{2B}$ receptor |
| **DNA Accesion Number:** | GenBank: NM_000676 |
| **Host Cell:** | HEK293 |
| **Resistance:** | Puromycin |
| **References:** | |

- ***P30192:*** 2 vials of 3 x $10^6$ proliferative cells
- ***P30192-DA***: 1 vial of 2.5 x $10^6$ division-arrested cells

| | |
|---|---|
| **Storage:** | Liquid Nitrogen |

## *Assay Briefly description*

Each vial of HiTSeeker ADORA2B contains HEK293 cells stably expressing human Adenosine A2B receptor with no tag.

Innoprot's HiTSeeker ADORA2B cell line has been designed to assay compounds or analyze their capability to modulate Adenosine A2B receptor. When the agonist binds to ADORA2B, a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (cAMP).

This cell line has been validated measuring cAMP increase in the cytosol. The high reproducibility of this assay allows monitoring ADORA2B activation process in High Throughput Screening.

## *About ADORA2B*

The gene encodes a protein which is one of several receptor subtypes for adenosine. The encoded protein is abundant in cecum, colon and bladder. A2B receptors are believed to play a role in the relaxation of smooth muscle in the vasculature and intestines. In addition, A2B receptor can negatively regulate monocyte and macrophage function. This receptor is responsible as well for stimulating mast cell mediator release. In fact, the A2B receptor is also expressed in lower levels in the lung, blood vessels, eye, median eminence and mast cells.

This protein also interacts with netrin-1, which is involved in axon elongation. The gene is located on chromosome 17, near the Smith-Magenis syndrome region.

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## Assay Characterization

Our expression plasmid contains the coding sequence of human ADORA2B protein. Our plasmid was transfected in HEK293 cells. Resistant clones were obtained by limit dilution and receptor gene expression was tested by RT-PCR using GAPDH as internal control (Fig.1).

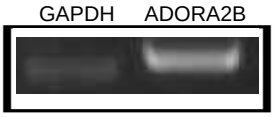


**Fig.1. ADORA2B and GAPDH housekeeping gene RT-PCR.**

## Validation of HiTSeeker ADORA2B

### cAMP production assay (Ec50= 1.16x10$^{-6}$M)

cAMP production was assessed using the cAMP dynamic 2 kit (Cisbio). This kit contains labelled cAMP (620 nm) and an anti-cAMP antibody (665nm). Between these molecules occurs a fluorescence transfer (FRET). Native cAMP produced by cells (due to the binding of an agonist to its specific receptor) competes with the labelled cAMP producing a decrease of FRET detected by HTRF technology.

The specific signal is inversely proportional to the concentration of native cAMP produced by the binding of the agonist to its receptor (Fig.2).

Fluorescence detection was recorded in a Multi-Mode Microplate Reader Synergy 2 from Biotek.

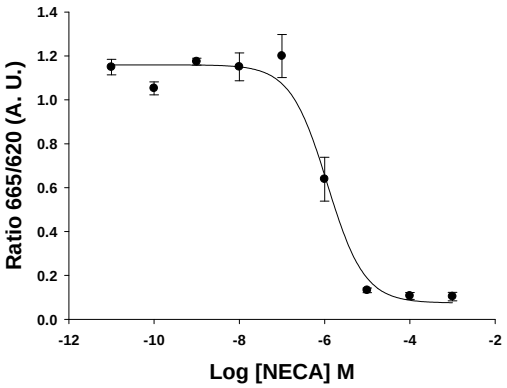


**Fig.2. ADORA2B dose response in cAMP assay.** Cells were treated with **NECA** concentrations ranging from 0 to 1 mM, n=4. The EC50 for **NECA** was 1.16x10$^{-6}$M. The cAMP assay was validated with a Z´= 084+/- 0.02 for High Content Screening.

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