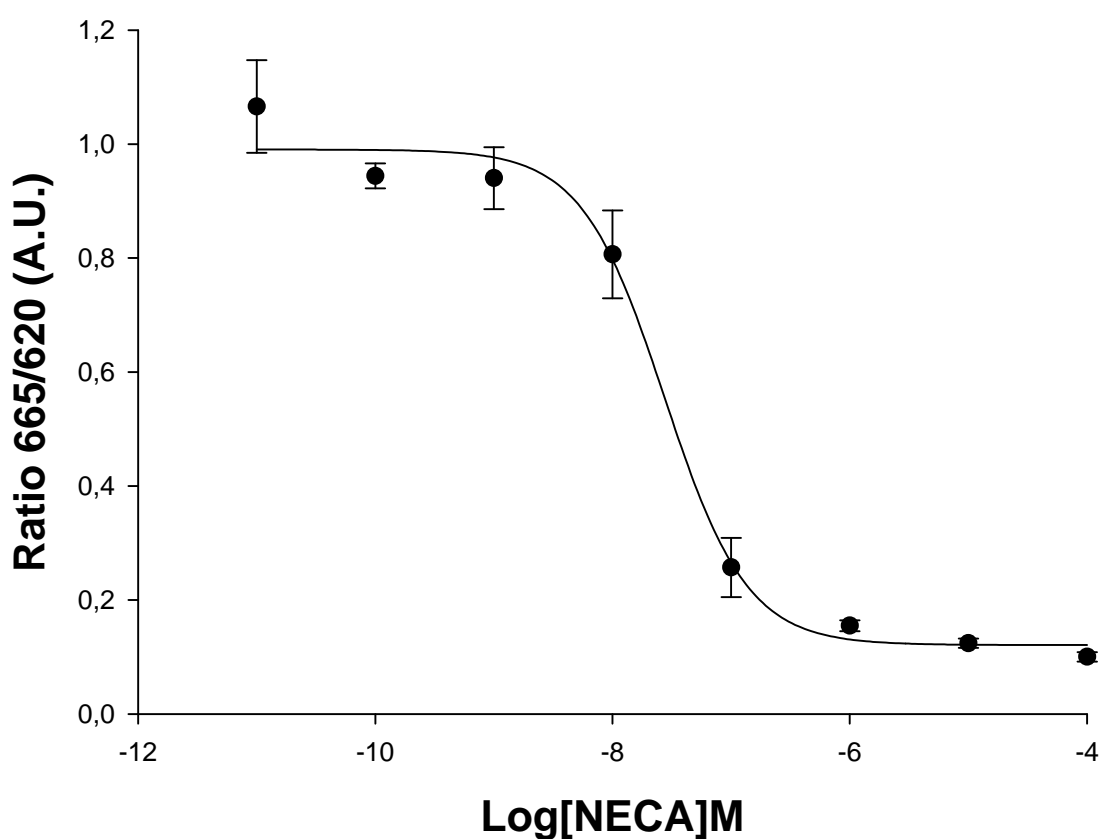


## HiTSeeker CELL LINES (LABEL-FREE GPCRS)

### - ADENOSINE RECEPTOR A2A (ADORA2A) CELL LINE -





**Product name:** ADORA2A /HEK293 cell line

**EC<sub>50</sub> NECA:** 2.75 x 10<sup>-8</sup>M

**Z':** 0.94 +/- 0.02

## - ADENOSINE RECEPTOR A<sub>2A</sub> CELL LINE -

<b>Product Name:</b>	ADORA2A/HEK293
<b>Official Full Name:</b>	Adenosine A <sub>2A</sub> receptor
<b>DNA Accession Number:</b>	BC013780
<b>Host Cell:</b>	HEK293
<b>Resistance:</b>	Puromycin
<b>References:</b>	

-  **P30191:** 2 vials of 3 x 10<sup>6</sup> proliferative cells
-  **P30191-DA:** 1 vial of 2.5 x 10<sup>6</sup> division-arrested cells

**Storage:** Liquid Nitrogen

### **Assay Briefly description**

Each vial of HiTSeeker ADORA2A contains HEK293 cells stably expressing human Adenosine A<sub>2A</sub> receptor with no tag.

Innoprot's HiTSeeker ADORA2A cell line has been designed to assay compounds or analyze their capability to modulate Adenosine A<sub>2A</sub> receptor. When the agonist binds to ADORA2A a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (cAMP).

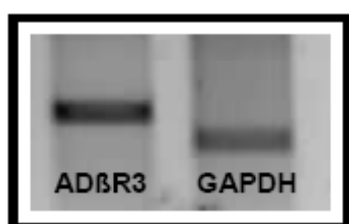
This cell line has been validated measuring cAMP increase in the cytosol. The high reproducibility of this assay allows monitoring ADORA2A activation process in High Throughput Screening.

### **About ADORA2A**

The gene encodes a protein which is one of several receptor subtypes for adenosine. The encoded protein is abundant in basal ganglia, vasculature, T lymphocytes, and platelets. A<sub>2A</sub> receptors are believed to play a role in regulating myocardial oxygen consumption and coronary blood flow. In addition, A<sub>2A</sub> receptor can negatively regulate overreactive immune cells, thereby protecting tissues from collateral inflammatory damage. The A<sub>2A</sub> receptor is responsible for regulating myocardial blood flow by vasodilating the coronary arteries, which increases blood flow to the myocardium, but may lead to hypotension. The A<sub>2A</sub> receptor is also expressed in the brain, where it has important roles in the regulation of glutamate and dopamine release, making it a potential therapeutic target for the treatment of conditions such as insomnia, pain, depression, drug addiction and Parkinson's disease

## **Assay Characterization**

Our expression plasmid contains the coding sequence of human ADORA2A 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. ADORA2A and GAPDH housekeeping gene RT-PCR.**

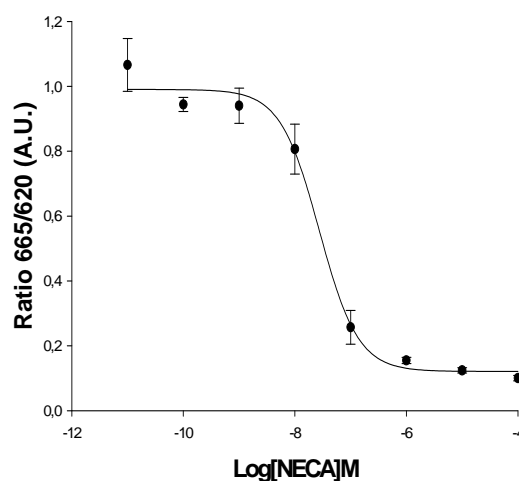
## **Validation of ADORA2A cell line**

### **cAMP production assay**

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.

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



**Fig.2. ADORA2A dose response in AMP<sub>c</sub> assay.**

Cells were treated with NECA concentrations ranging from 0 to 100  $\mu$ M, n=3. The EC<sub>50</sub> for NECA was  $\sim 2.75 \times 10^{-8}$  M. The cAMP assay was validated with a  $Z' = 0.943$  for High Content Screening.