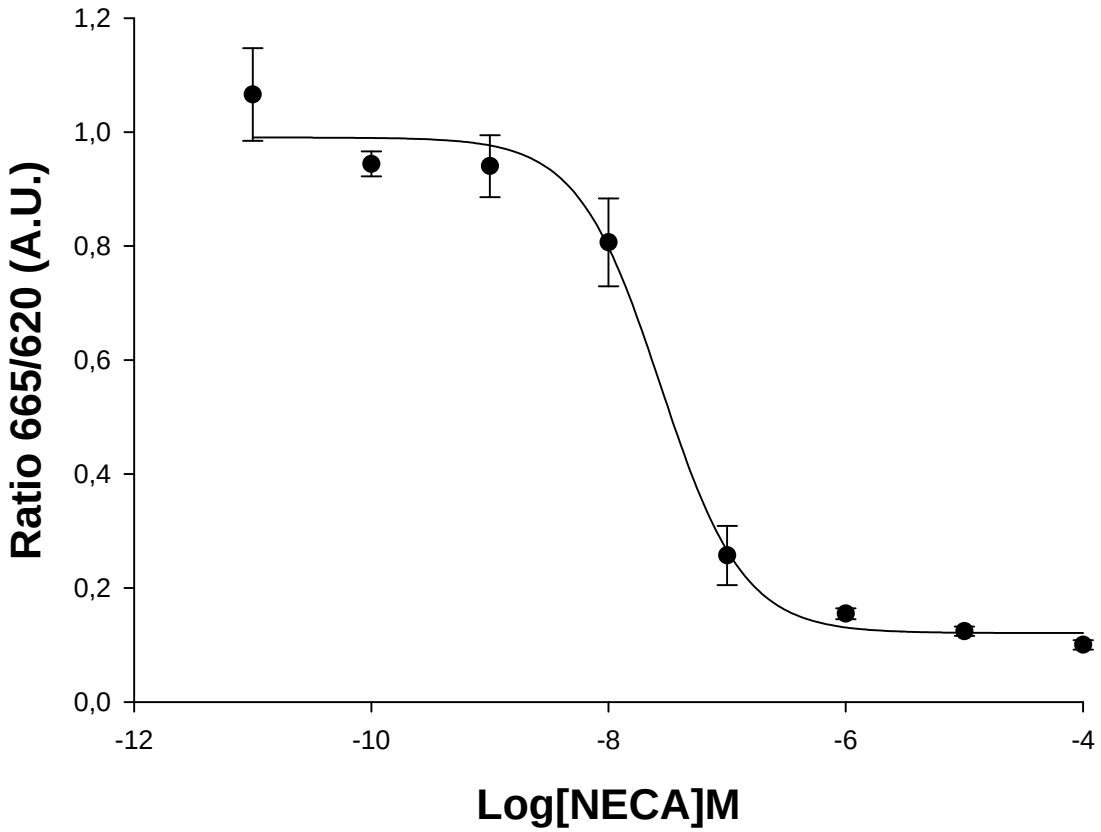
REF: P30191

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- ADENOSINE RECEPTOR A2A (ADORA2A) CELL LINE -



Product name: ADORA2A /HEK293 cell line

Ec_{50} NECA: 2.75 x 10^{-8}M

Z´: 0.94 +/- 0.02

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- ADENOSINE RECEPTOR A2A CELL LINE -

Product Name:	ADORA2A/HEK293
Official Full Name:	Adenosine A_{2A} receptor
DNA Accesion Number:	BC013780
Host Cell:	HEK293
Resistance:	Puromycin
References:	
	P30191: 2 vials of 3 x 10^6 proliferative cells
	P30191-DA: 1 vial of 2.5 x 10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker ADORA2A contains HEK293 cells stably expressing human Adenosine A_{2A} receptor with no tag.

Innoprot's HiTSeeker ADORA2A cell line has been designed to assay compounds or analyze their capability to modulate Adenosine A_{2A} 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. A2A receptors are believed to play a role in regulating myocardial oxygen consumption and coronary blood flow. In addition, A2A receptor can negatively regulate overreactive immune cells, thereby protecting tissues from collateral inflammatory damage. The A2A 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 A2A 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

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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).

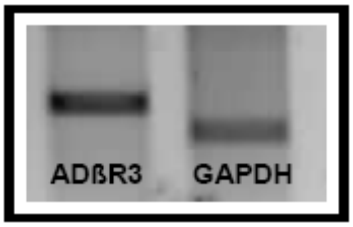

Fig.1. ADORA2A and GAPDH housekeeping gene RT-PCR.

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.

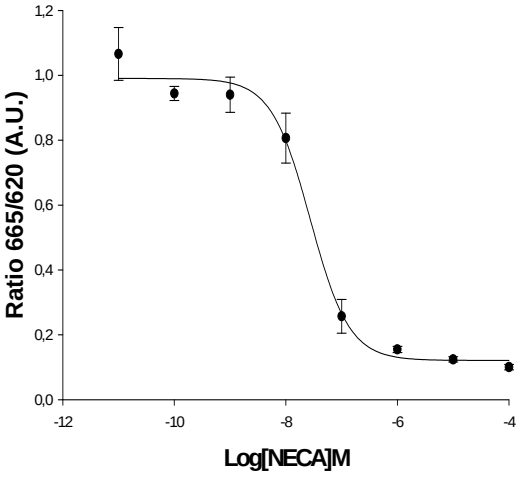

Fig.2. ADORA2A dose response in AMP_c assay. Cells were treated with NECA concentrations ranging from 0 to 100 µM, n=3. The EC50 for NECA was ~$2.75x10^{-8}$M. The cAMP assay was validated with a Z´= 0.943 for High Content Screening.

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