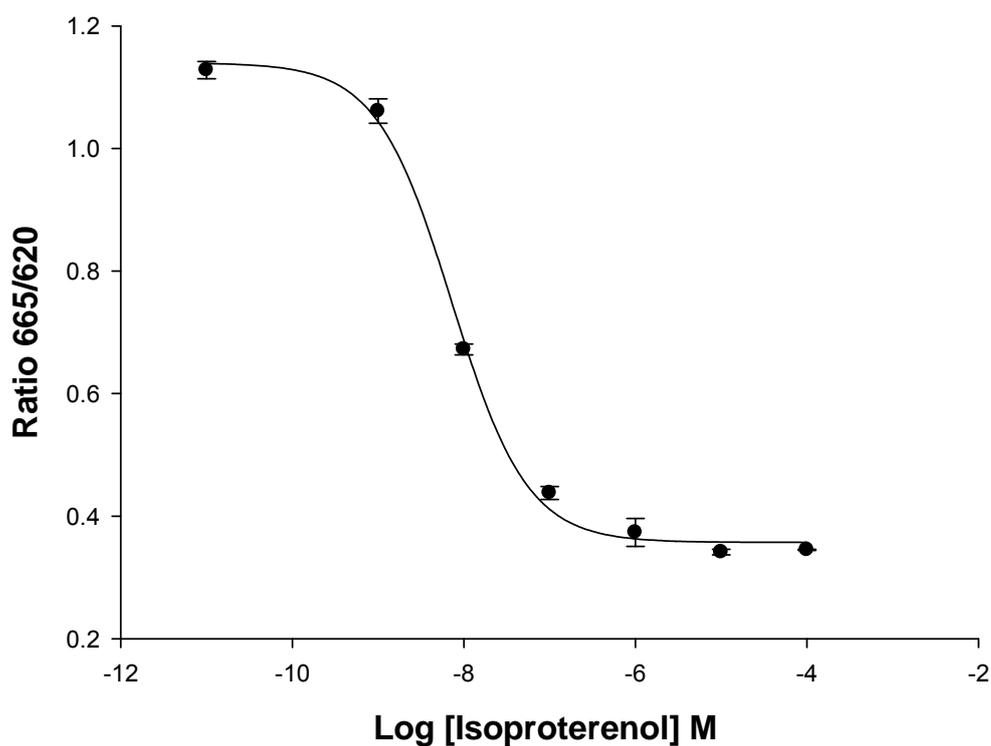


## HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- ADRENERGIC  $\beta_2$  RECEPTOR CELL LINE -



**Product name:** ADR $\beta_2$  ( $\beta_2$  adrenoreceptor) /HEK293 cell line

**Ec<sub>50</sub> Isoproterenol:**  $7.27 \times 10^{-9}$  M

**Z':** 0.94 $\pm$  0.02

## - ADRENERGIC $\beta_2$ RECEPTOR CELL LINE -

---

<b>Product Name:</b>	ADRB $\beta_2$ ( $\beta_2$ adrenoreceptor)/HEK293
<b>Official Full Name:</b>	beta-2 adrenergic receptor
<b>DNA Accesion Number:</b>	GenBank: NM_000024.3
<b>Host Cell:</b>	HEK293
<b>Format:</b>	2 cryopreserved vials
<b>Resistance:</b>	Puromycin
<b>Size:</b>	P30125: 2 vials of $3 \times 10^6$ proliferative cells P30125-DA: 1 vial of $2.5 \times 10^6$ division-arrested cells
<b>Storage:</b>	Liquid Nitrogen

---

### **Assay Briefly description**

Each vial of HiTSeeker ADR $\beta_2$  contains HEK293 cells stably expressing human beta 2 adrenergic receptor with no tag.

Innoprot's HiTSeeker ADR $\beta_2$  cell line has been designed to assay compounds or analyze their capability to modulate adrenergic  $\beta_2$  receptor. When the agonist binds to ADR $\beta_2$ , a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (Calcium).

This cell line has been validated measuring cAMP increase in the cytosol. The high reproducibility of this assay allows monitoring ADR $\beta_2$  activation process in High Throughput Screening.

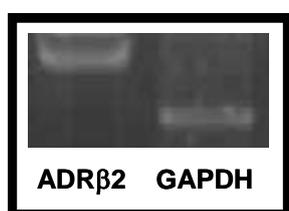
### **About ADR $\beta_2$**

This gene encodes beta-2-adrenergic receptor which is a member of the G protein-coupled receptor superfamily. The adrenergic receptors are a class of G protein-coupled receptors that are targets of the catecholamines, especially noradrenaline and adrenaline. Although dopamine is a catecholamine, its receptors are in a different category.

This receptor is directly associated with one of its ultimate effectors, the class C L-type calcium channel. This receptor-channel complex also contains a G protein, an adenylyl cyclase, a cAMP-dependent kinase and the counterbalancing phosphatase, PP2A. Many cells possess these receptors, and binding of an agonist will generally cause a sympathetic response. Different polymorphic forms, point mutations, and/or downregulation of this gene are associated with nocturnal asthma, obesity and type 2 diabetes.

## Assay Characterization

Our expression plasmid contains the coding sequence of human ADR $\beta$ 2 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. ADR $\beta$ 2 and GAPDH housekeeping gene RT-PCR.**

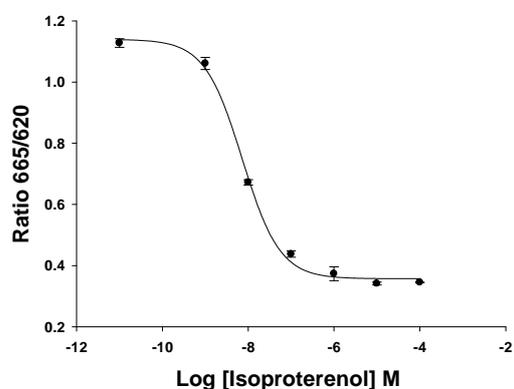
## Validation of ADR $\beta$ 2 cell line

### **cAMP production assay (EC<sub>50</sub>=7.27x10<sup>-9</sup> 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.

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



**Fig.2. ADR $\beta$ 2 dose response in cAMP assay.**

Cells were treated with **Isoproterenol** concentrations ranging from 0 to 100  $\mu$ M, n=35. The EC<sub>50</sub> for **Isoproterenol** was  $\sim$ 7.27x10<sup>-9</sup>M. The cAMP assay was validated with a Z' = 0.94 $\pm$  0.02 for High Throughput Screening.