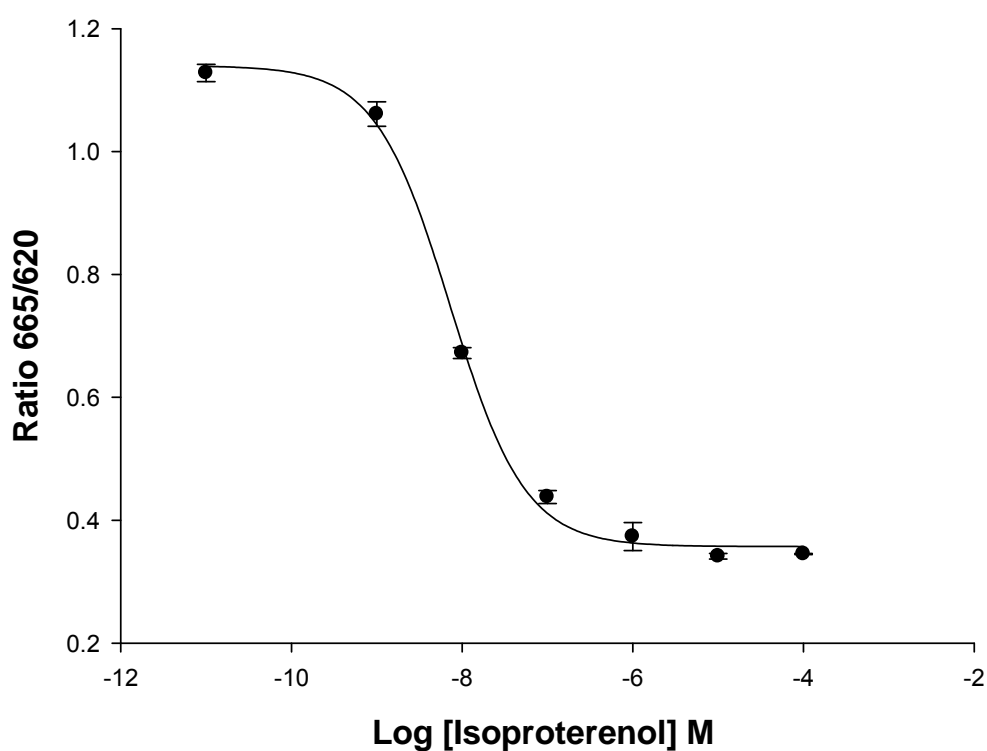


HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- ADRENERGIC β_2 RECEPTOR CELL LINE -



Product name: ADR β_2 (β_2 adrenoreceptor) /HEK293 cell line

Ec₅₀ Isoproterenol: 7.27×10^{-9} M

Z': 0.94 \pm 0.02

- ADRENERGIC β_2 RECEPTOR CELL LINE -

| | |
|------------------------------|--|
| Product Name: | ADRB β_2 (β_2 adrenoreceptor)/HEK293 |
| Official Full Name: | beta-2 adrenergic receptor |
| DNA Accession Number: | GenBank: NM_000024.3 |
| Host Cell: | HEK293 |
| Format: | 2 cryopreserved vials |
| Resistance: | Puromycin |
| Size: | P30125: 2 vials of 3×10^6 proliferative cells P30125-DA: 1 vial of 2.5×10^6 division-arrested cells |
| Storage: | Liquid Nitrogen |

Assay Briefly description

Each vial of HiTSeeker ADR β_2 contains HEK293 cells stably expressing human beta 2 adrenergic receptor with no tag.

InnoproT's HiTSeeker ADR β_2 cell line has been designed to assay compounds or analyze their capability to modulate adrenergic β_2 receptor. When the agonist binds to ADR β_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 β_2 activation process in High Throughput Screening.

About ADR β_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 β 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).

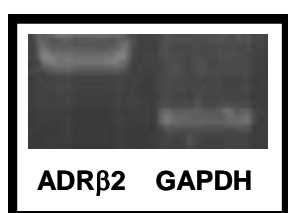


Fig.1. ADR β 2 and GAPDH housekeeping gene RT-PCR.

Validation of ADR β 2 cell line

cAMP production assay (EC₅₀=7.27x10⁻⁹ 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.

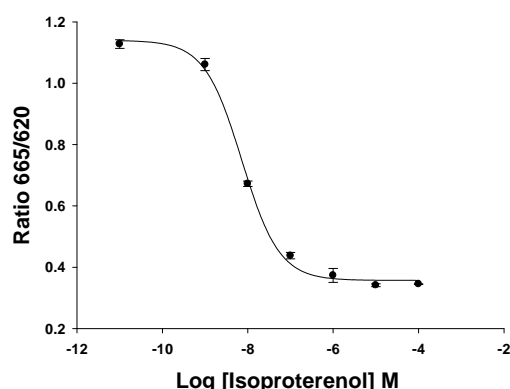


Fig.2. ADR β 2 dose response in cAMP assay.

Cells were treated with **Isoproterenol** concentrations ranging from 0 to 100 μ M, n=35. The EC₅₀ for **Isoproterenol** was 7.27x10⁻⁹M. The cAMP assay was validated with a Z' = 0.94 +/- 0.02 for High Throughput Screening.