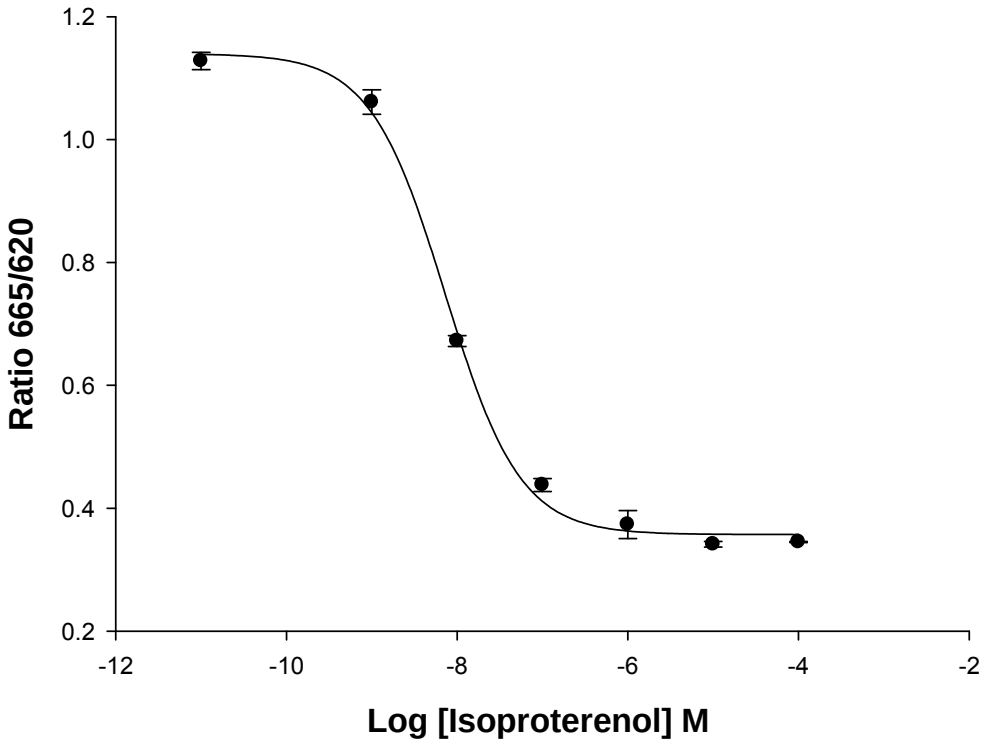
REF: P30125

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- ADRENERGIC ß2 RECEPTOR CELL LINE -



Product name: ADRß2 (β_2 adrenoreceptor) /HEK293 cell line

Ec_{50} Isoproterenol: $7.27x10^{-9}$ M

Z´: 0.94+/- 0.02

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

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- ADRENERGIC ß2 RECEPTOR CELL LINE -

Product Name:	ADRß2 (β_2 adrenoreceptor)/HEK293
Official Full Name:	beta-2 adrenergic receptor
DNA Accesion Number:	GenBank: NM_000024.3
Host Cell:	HEK293
Format:	2 cryopreserved vials
Resistance:	Puromycin
Size:	P30125: 2 vials of $3x10^6$ proliferative cells
	P30125-DA: 1 vial of $2.5x10^6$ division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker ADRß2 contains HEK293 cells stably expressing human beta 2 adrenergic receptor with no tag.

Innoprot's HiTSeeker ADRß2 cell line has been designed to assay compounds or analyze their capability to modulate adrenergic ß2 receptor. When the agonist binds to ADRß2, 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 ADRß2 activation process in High Throughput Screening.

About ADRß2

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.

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Assay Characterization

Our expression plasmid contains the coding sequence of human ADRß2 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).

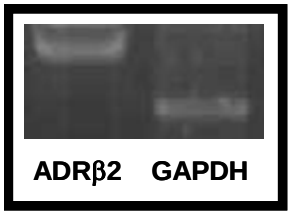

Fig.1. ADRß2 and GAPDH housekeeping gene RT-PCR.

Validation of ADRß2 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.

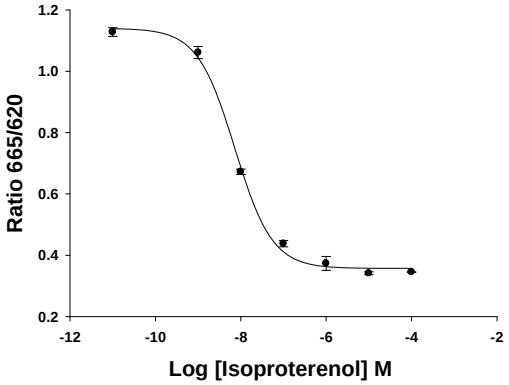

Fig.2.ADRß2 dose response in cAMP assay. Cells were treated with **Isoproterenol** concentrations ranging from 0 to 100 µM, n=35. The EC50 for **Isoproterenol** was ~7.27x10^{-9}M. The cAMP assay was validated with a Z´= 0.94+/- 0.02 for High Throughput Screening.

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