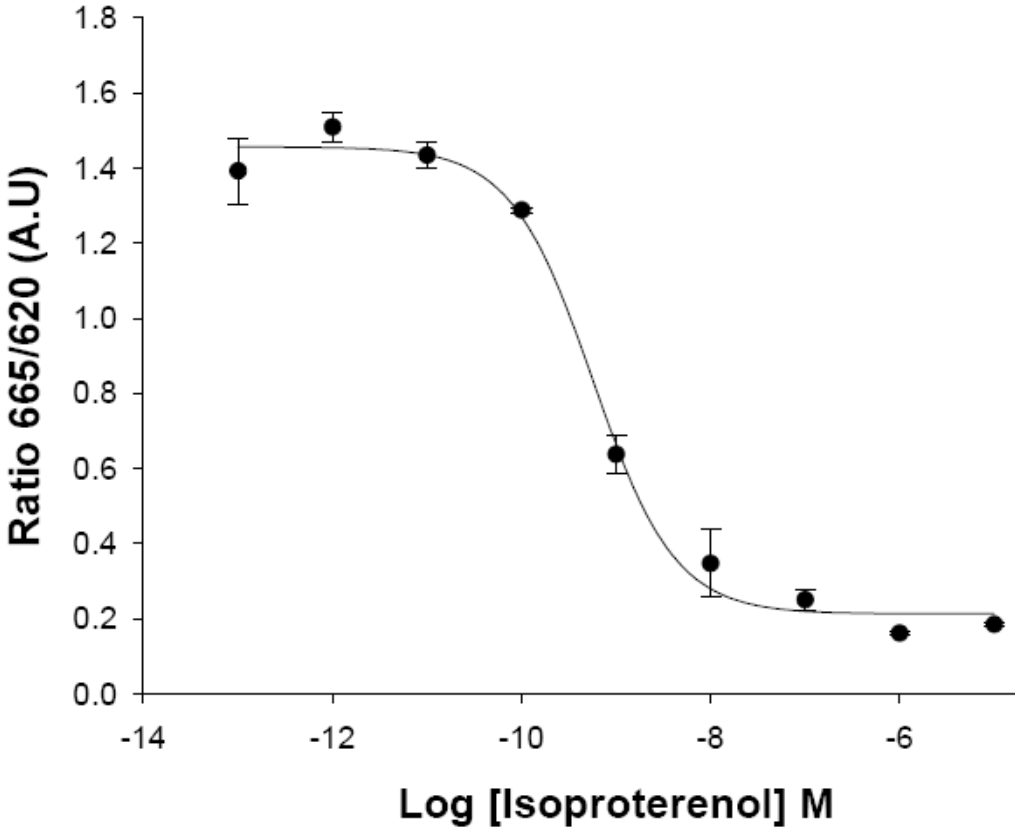
REF: P30101

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- ADRENERGIC ß3 RECEPTOR CELL LINE -



Product name: ADRß3 (β_3 adrenoreceptor) /HEK293 cell line

Ec_{50} Isoproterenol: $5.67x10^{-10}$M

Z´: 0.77+/- 0.02

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- ADRENERGIC ß3 RECEPTOR CELL LINE -

Product Name:	ADRß3 (β_3 adrenoreceptor)/HEK293
Official Full Name:	beta-3 adrenergic receptor
DNA Accesion Number:	GenBank: AY487247
Host Cell:	HEK293
Format:	Cryopreserved vials
Resistance:	Puromycin
Size:	*P30101*: 2 vials of 3 x 10^6 proliferative cells
	P30101-DA: 1 vial of 2.5x10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker ADRß3 contains HEK293 cells stably expressing human beta 3 adrenergic receptor with no tag.

HiTSeeker ADRß3 cell line has been designed to assay compounds or analyze their capability to modulate adrenergic ß3 Receptor. When the agonist binds to ADRß3 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 ADRß3 activation process in High Throughput Screening.

About ADRß3

The protein encoded by this gene belongs to the family of beta adrenergic receptors, which mediate catecholamine-induced activation of adenylate cyclase through the action of G proteins.

ADRß3 mediates in lipolysis in the adipose tissue and in thermogenesis in the skeletal muscle.

Some ß3 agonists have shown antidepressant effects in animal studies.

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Assay Characterization

Our expression plasmid contains the coding sequence of human ADRß3 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).

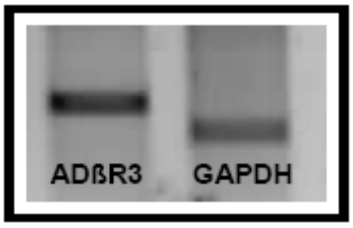

Fig.1. ADRß3 and GAPDH housekeeping gene RT-PCR.

Validation of ADRß3 cell line

cAMP production assay (Ec50=5.67x10⁻¹⁰M)

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.

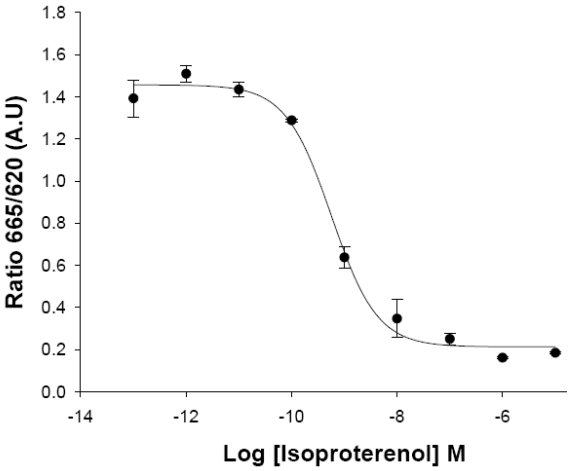

Fig.2.ADRß3 **dose response in calcium assay.** Cells were treated with **Isoproterenol** concentrations ranging from 0 to 10 µM, n=5. The EC50 for **Isoproterenol** was ~5.67x10^{-10}M. The cAMP assay was validated with a Z´= 0.77+/- 0.02 for High Throughput Screening.

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