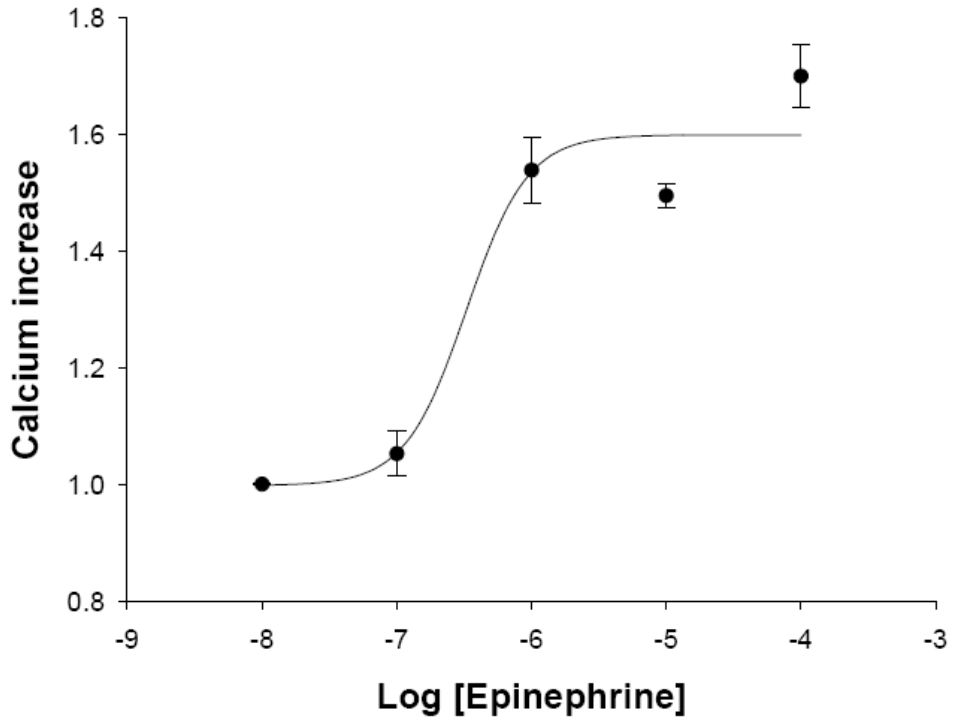
REF: P30141

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

-ALPHA-1B ADRENERGIC RECEPTOR CELL LINE -



Product name: ADRA1B (α_{1B} adrenoreceptor)/U2OS cell line

Ec_{50} Epinephrine: 3.28 x 10^{-7} M

Z´: 0.76+/- 0.02

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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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- ALPHA-1B ADRENERGIC RECEPTOR CELL LINE -

Product Name:	ADRA1B (α_{1B} adrenoreceptor)/U2OS
Official Full Name:	Alpha-1B adrenergic Receptor
DNA Accession Number:	GenBank: NM_000679
Host Cell:	U2OS
Format:	Cryopreserved vials
Resistance:	Puromycin
Size:	*P30141*: 2 vials of 3 x 10^6 proliferative cells *P30141-DA*: 1 vial of 2.5x10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

HiTSeeker ADRA1B contains U2OS cells stably expressing human alpha-1B adrenergic receptor with no tag.

HiTSeeker ADRA1B cell line has been designed to assay compounds or analyze their capability to modulate Alpha-1B adrenergic Receptor. When the agonist binds to ADRA1B a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (Calcium).

This cell line has been validated measuring calcium increase in the cytosol. The high reproducibility of this assay allows monitoring ADRA1B activation process in High Throughput Screening.

About ADRA1B

Alpha-1-adrenergic receptors (alpha-1-ARs) are members of the G protein-coupled receptor superfamily. There are 3 alpha-1-AR subtypes: alpha-1A, -1B and -1D, all of which signal through the Gq/11 family of G-proteins.

The α(1)-adrenergic receptor (AR) subtypes (α(1a), α(1b), and α(1d)) mediate some physiological effects of epinephrine and norepinephrine.

The α_1-AR subtypes are expressed in several organs like brain or heart in which they modulate a diversity of functional effects such as modulation of neurotransmission, vasoconstriction, etc.

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Assay Characterization

Our expression plasmid contains the coding sequence of human ADRA1B protein. Our plasmid was transfected in U2OS 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.ADRA1B and GAPDH housekeeping gene RT-PCR.

Validation of ADRA1B cell line

Calcium assay (Ec50 = 3.28 x 10^{-7}M)

A typical fluorescent calcium assay was performed using Fura-2/AM ratiometric. Calcium increase inside the cell was measured using the ratio of the fluorescence from Fura2 bound and not bound to the ion. Image acquisition was performed using a "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Cells were incubated with Fura2-AM and treated with increasing Epinephrine concentrations.

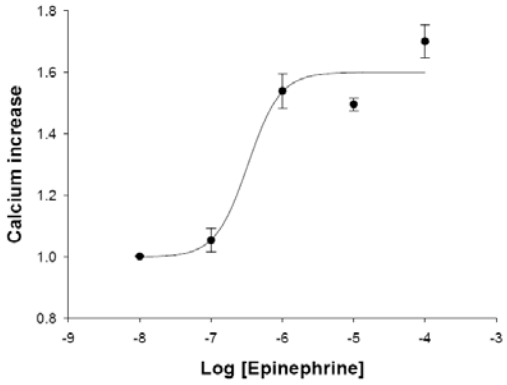

Fig.2. ADRA1B **dose response in calcium assay.** Cells were treated with **Epinephrine** concentrations ranging from 0 to 100 µM, n=4. The EC50 for **Epinephrine** was ~**3.28 x10^{-7}M**. The calcium assay was validated with a Z´= 0.76+/- 0.02 for High Content Screening.

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