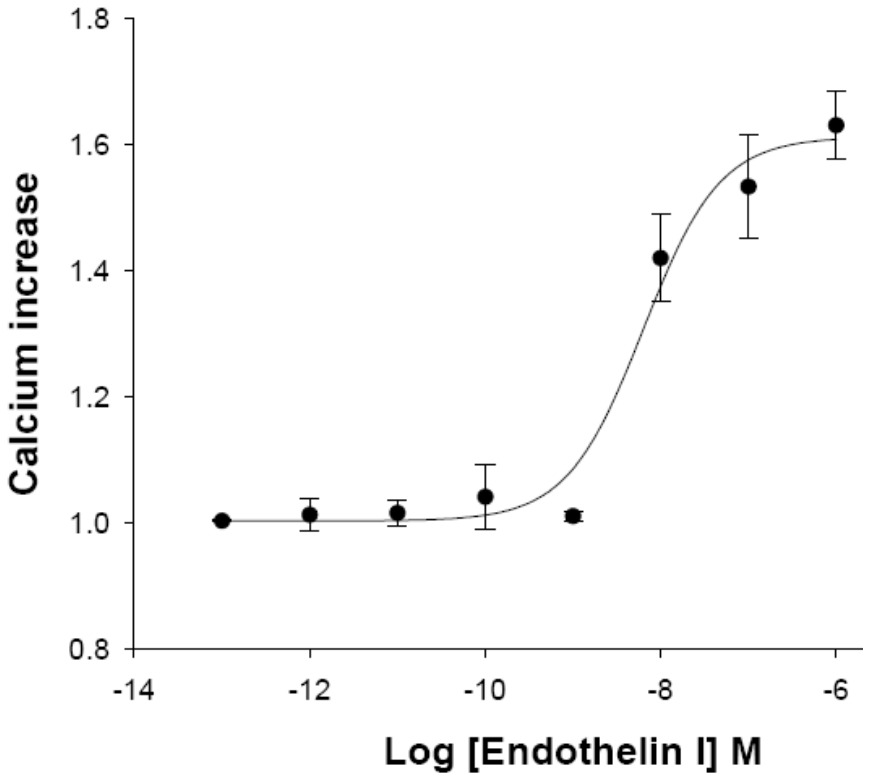
REF: P30152

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

-ENDOTHELIN RECEPTOR TYPE B (ET_B) CELL LINE -



Product name: ET_B (EDNRB) /U2OS cell line

Ec_{50} Endothelin 1: 6.35x 10^{-9} M

Z´: 0.74+/- 0.02

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- ENDOTHELIN RECEPTOR TYPE B U2OS CELL LINE -

Product Name:	ET_B (EDNRB)/U2OS
Official Full Name:	Endothelin receptor type B
DNA Accession Number:	GenBank: AY275463
Host Cell:	U2OS
Format:	2 cryopreserved vials
Resistance:	G418
Size:	> 3 x 10^6 cells / vial
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker ET_B/U2OS contains U2OS cells stably expressing human Endothelin receptor type B (ET_B) with no tag.

Innoprot's HiTSeeker ET_B cell line has been designed to assay compounds or analyze their capability to modulate Endothelin receptor type B. When the agonist binds to ET_B 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 ET_B activation process in High Throughput Screening.

About ET_B

The endothelins and their receptors are referred as the endothelin (ET) axis and this axis has been implicated in diverse tumours.

Endothelin B receptor (ET_B) may oppose cancer advance by helping apoptosis and clearing ET-1; however, it has recently been involved in the progress of some tumour as melanomas or oligodendrogliomas.

The multigenic disorder, Hirschsprung disease type 2, is due to mutation in endothelin receptor type B gene.

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Assay Characterization

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

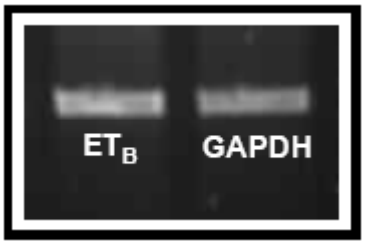

Fig.1. ET_B and GAPDH housekeeping gene RT-PCR.

Validation of HiTSeeker ET_B

Calcium assay (Ec50 = 6.35 x 10^{-9}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 Endothelin 1 concentrations.

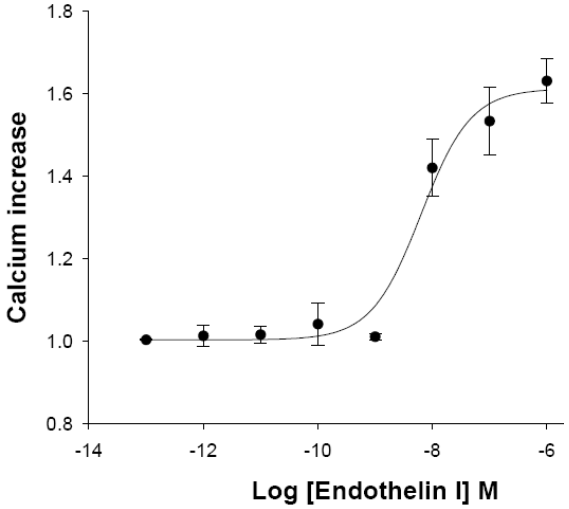

Fig.2. *HiTSeeker* ET_B dose response in calcium assay. Cells were treated with **Endothelin 1** concentrations ranging from 0 to 1 µM, n=5. The EC50 for **Endothelin 1** was ~**6.35x10^{-9}M**. The calcium assay was validated with a Z´= 0.74+/- 0.02 for High Content Screening.

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