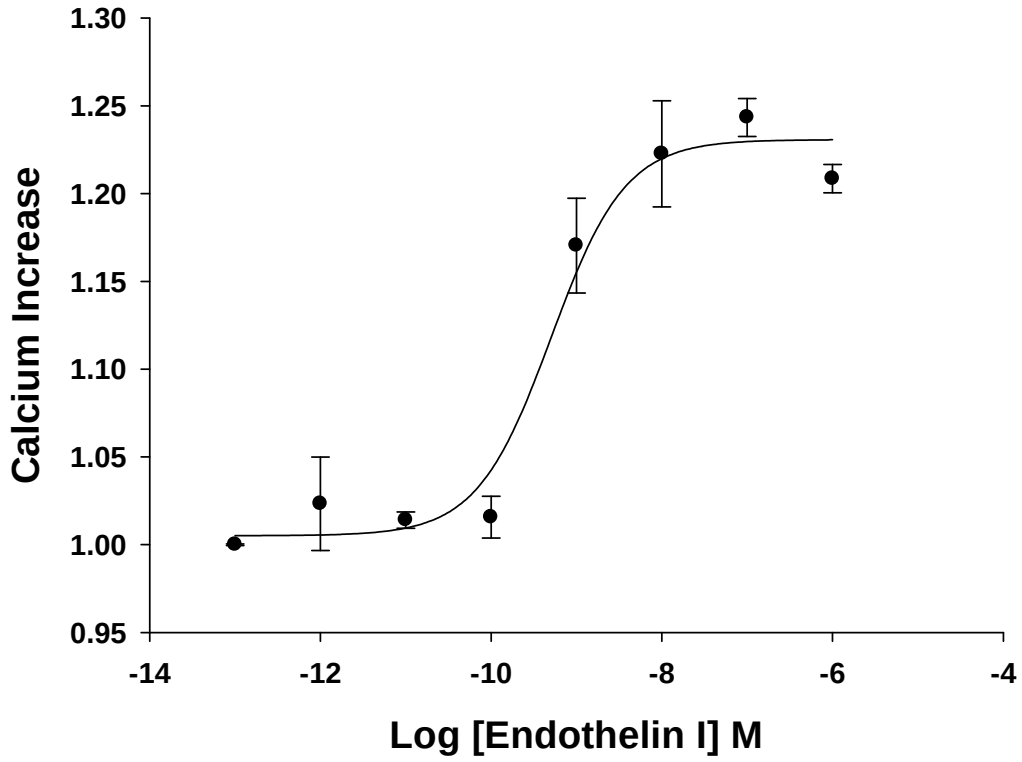
REF: P30151

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

-ENDOTHELIN RECEPTOR TYPE A (ET_A) CELL LINE -



Product name: ET_A (EDNRA) /U2OS cell line

Ec_{50} Endothelin 1: 5.04x 10^{-10} M

Z´: 0.88+/- 0.02

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Innoprot
Speeding up drug discovery

REF: P30151

- ENDOTHELIN RECEPTOR TYPE A U2OS CELL LINE -

Product Name:	HiTSeeker ET_A (EDNRA)
Official Full Name:	Endothelin receptor type A
DNA Accession Number:	GenBank: AY275462
Host Cell:	U2OS
Format:	2 cryopreserved vials
Resistance:	G418
References:	
P30151	2 vials of 3 x 10^6 proliferative cells
P30151-DA	1 vial of 2.5 x 10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker EDNRA contains U2OS cells stably expressing human Endothelin receptor type A (ET_A) with no tag.

Innoprot's HiTSeeker EDNRA (ET_A) cell line has been designed to assay compounds or analyze their capability to modulate Endothelin receptor type A. When the agonist binds to ET_A, 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_A activation process in High Throughput Screening.

About ET_A

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

Endothelin A receptor (ET_A) favours blood presure by increasing vasoconstriction and sodium retention after ET binding1; this is why it is more abundant in smooth muscle cells of blood vessels.

Polymorphisms in this gene have been linked to migraine headache resistance. Alternative splicing results in multiple transcript variants.

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Assay Characterization

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

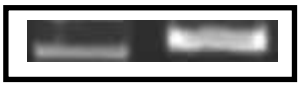

Fig.1. GAPDH and ET_A housekeeping gene RT-PCR.

Validation of ET_A cell line

Calcium assay (Ec50 = 5.04 x 10^{-10}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.

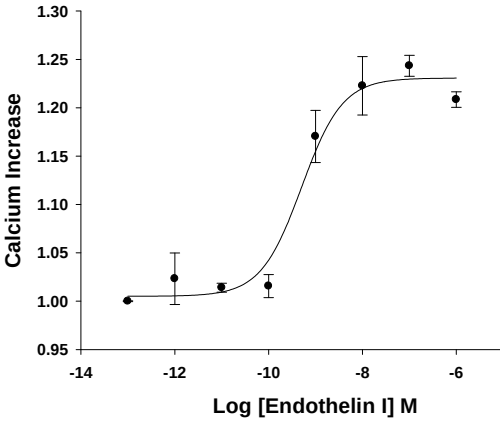

Fig.2. ET_A dose response in calcium assay. Cells were treated with **Endothelin 1** concentrations ranging from 0 to 1 µM, n=4. The EC50 for **Endothelin 1** was ~**5.04x10^{-10}M**. The calcium assay was validated with a Z´= 0.88+/- 0.02 for High Content Screening.

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