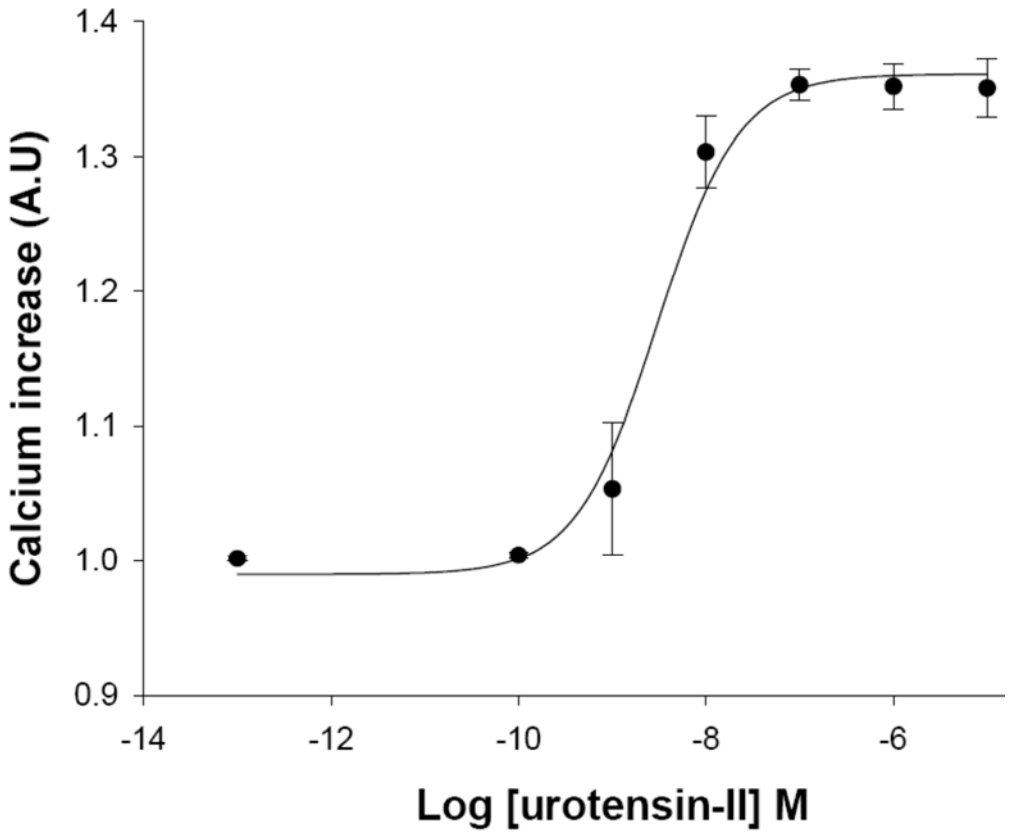
REF: P30424

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- UROTENSIN-II RECEPTOR (UT) CELL LINE -



Product name: UT (UTS2R) /U2OS cell line

Ec_{50} urotensin-II: 3.02 x 10^{-9} M

Z´: 0.80+/- 0.02

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- UROTENSIN-II RECEPTOR (UT) CELL LINE -

Product Name:	HiTSeeker UTS2R
Official Full Name:	Urotensin-II receptor
DNA Accession Number:	GenBank: NM_018949
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 UTS2R 3 million U2OS cells stably expressing human Urotensin-II receptor (UT) with no tag.

HiTSeeker UTS2R cell line has been designed to assay compounds or analyze their capability to modulate Urotensin-II receptor. When the agonist binds to UT 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 UT activation process in High Throughput Screening.

About UTS2R

Urotensin-II receptor (UTS2R) is a G-protein coupled receptor which binds the peptide hormone urotensin-II.

Urotensin II receptor (UTS2R) is one of the most potent vasoconstrictors.

UTS2R may play an important role in cardiovascular regulation.

UTS2R could be an interesting target for new treatment for cardiorenal diseases.

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Assay Characterization

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

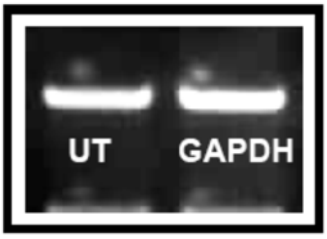

Fig.1.UT and GAPDH housekeeping gene RT-PCR.

Validation of UT *cell line*

Calcium assay (EC_{50} = 3.02 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 urotensin-II concentrations.

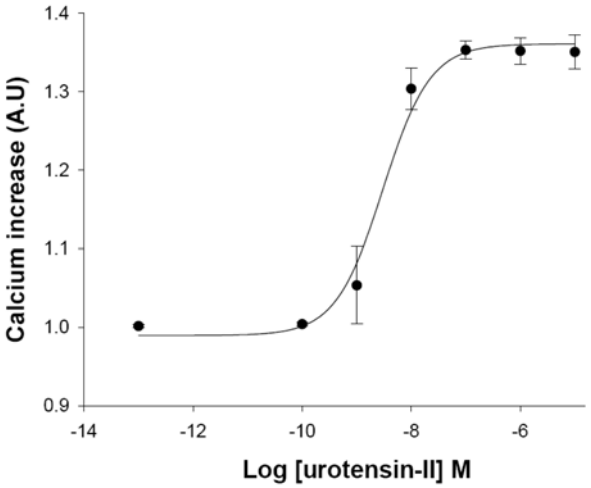

Fig.2. UT dose response in calcium assay. Cells were treated with urotensin-II concentrations ranging from 0 to 10 µM, n=6. The EC_{50} for Vasopressin was ~$3.02x10^{-9}$M. The calcium assay was validated with a Z´= 0.80+/- 0.02 for High Content Screening.

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