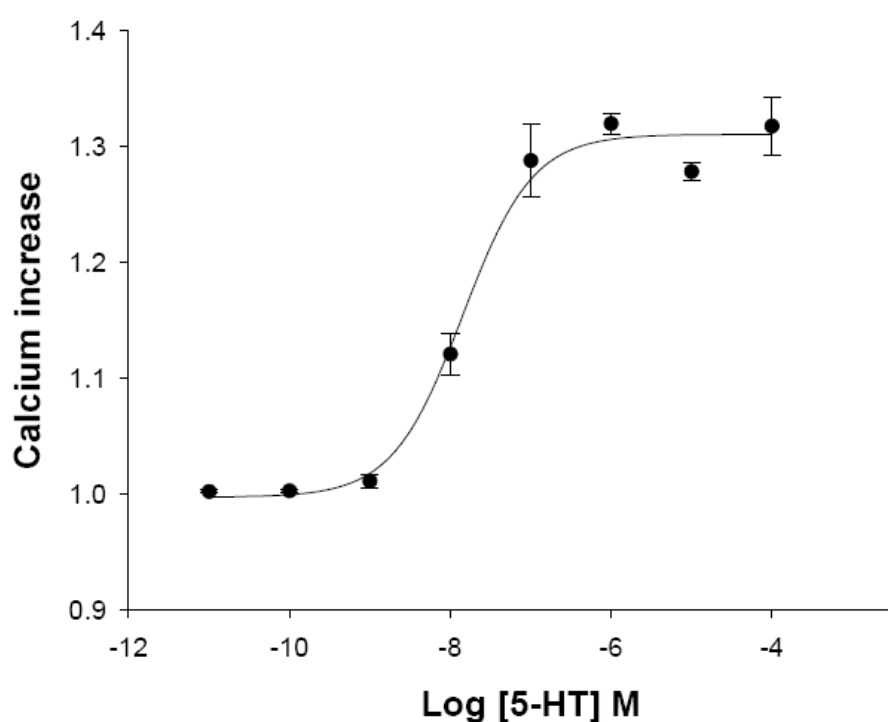


## **HiTSeeker CELL LINES (LABEL-FREE GPCRS)**

### **- 5-HYDROXYTRYPTAMINE RECEPTOR 2A (HTR2A) CELL LINE -**



**Product name:** HTR2A (5-HT<sub>2A</sub>) /U2OS cell line

**EC<sub>50</sub> 5-HT:** 1.4x 10<sup>-8</sup> M

**Z':** 0.75+/- 0.02

## - 5-HYDROXYTRYPTAMINE RECEPTOR 2A (HTR2A) U2OS CELL LINE -

<b>Product Name:</b>	HTR2A (5-HT <sub>2A</sub> )/U2OS
<b>Official Full Name:</b>	5-Hydroxytryptamine receptor 2A
<b>DNA Accession Number:</b>	GenBank: NM_000621
<b>Host Cell:</b>	U2OS
<b>Format:</b>	2 cryopreserved vials
<b>Resistance:</b>	G418
<b>Size:</b>	> 3 x 10 <sup>6</sup> cells / vial
<b>Storage:</b>	Liquid Nitrogen

### **Assay Briefly description**

HiTSeeker HTR2A/U2OS contains U2OS cells stably expressing human 5-Hydroxytryptamine receptor 2A (HTR2A) with no tag.

Innoprot's HiTSeeker HTR2A cell line has been designed to assay compounds or analyze their capability to modulate 5-Hydroxytryptamine receptor 2A. When the agonist binds to HTR2A 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 HTR2A activation process in High Throughput Screening.

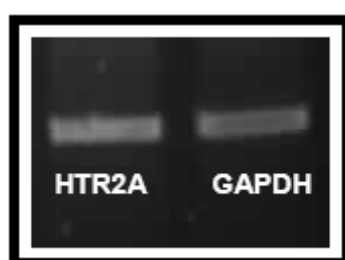
### **About HTR2A**

The serotonin receptors, also known as 5-hydroxytryptamine receptors or 5-HT receptors are a group of G protein-coupled receptors (GPCRs) found in the central and peripheral nervous systems. They mediate both excitatory and inhibitory neurotransmission. The neurotransmitter serotonin acts as their natural ligand.

The 5-hydroxytryptamine receptor 2A (5-HT<sub>2A</sub>) has been involved in diverse psychiatric disorders like schizophrenia, mood disorders, anxiety disorders, obsessive-compulsive disorder, eating disorders, and Alzheimer's disease.

## **Assay Characterization**

Our expression plasmid contains the coding sequence of human HTR2A 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).



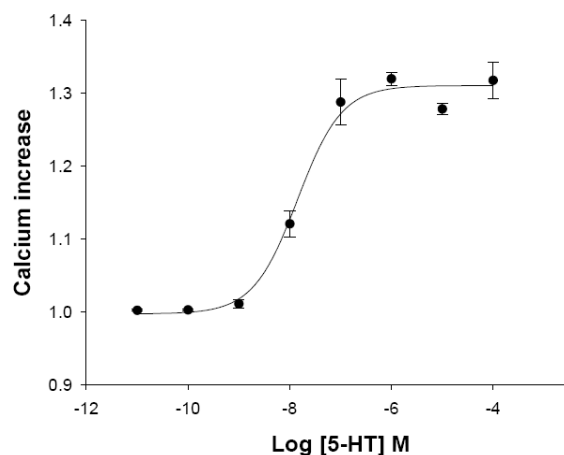
**Fig.1. HTR2A and GAPDH housekeeping gene RT-PCR.**

## **Validation of HTR2A cell line**

### **Calcium assay ( $EC_{50} = 1.41 \times 10^{-8}M$ )**

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 5-HT concentrations.



**Fig.2. HTR2A dose response in calcium assay.**

Cells were treated with 5-HT concentrations ranging from 0 to 100  $\mu M$ ,  $n=5$ . The  $EC_{50}$  for 5-HT was  $1.41 \times 10^{-8}M$ . The calcium assay was validated with a  $Z' = 0.75 \pm 0.02$  for High Content Screening.