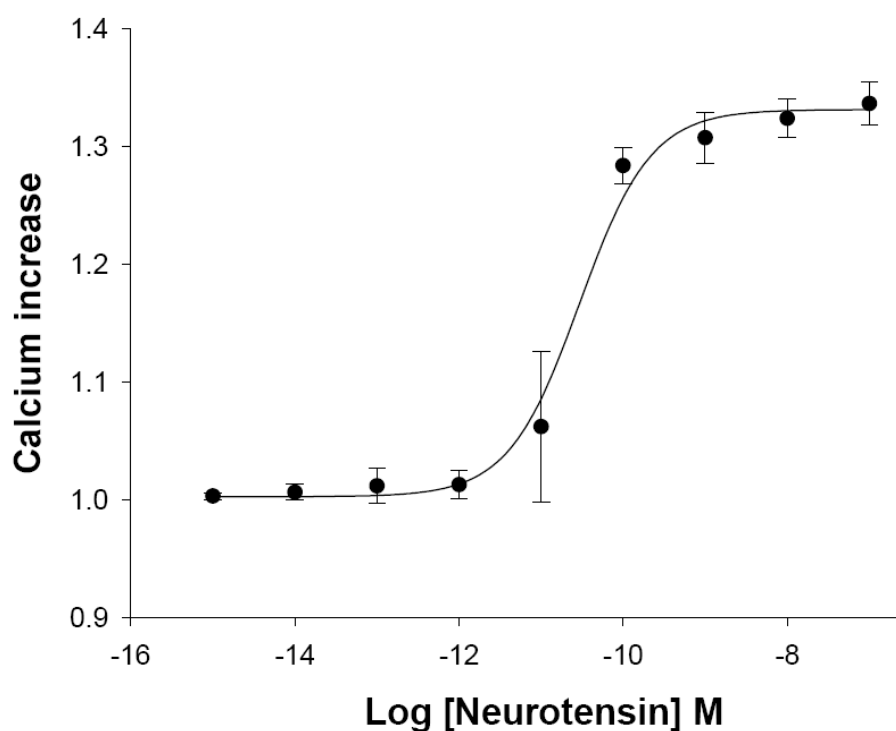


## **HiTSeeker CELL LINES (LABEL-FREE GPCRS)**

### **- NEUROTENSIN RECEPTOR 1 (NTSR1) CELL LINE -**



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**Product name:** NTSR1 (NTR) /U2OS cell line

**Ec<sub>50</sub> Neurotensin:**  $2.94 \times 10^{-11}$  M

**Z':** 0.80+/- 0.02

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## - NEUROTENSIN RECEPTOR 1 (NTSR1) U2OS CELL LINE -

<b>Product Name:</b>	NTSR1 (NTR)/U2OS
<b>Official Full Name:</b>	Neurotensin receptor type 1
<b>DNA Accession Number:</b>	GenBank: AY429106
<b>Host Cell:</b>	U2OS
<b>Format:</b>	2 cryopreserved vials
<b>Resistance:</b>	Puromycin
<b>References:</b>	
	 <b>P30187:</b> 2 vials of $3 \times 10^6$ proliferative cells
	 <b>P30187-DA:</b> 1 vial of $2 \times 10^6$ division-arrested cells
<b>Storage:</b>	Liquid Nitrogen

### **Assay Briefly description**

Each vial of HiTseeker NTSR1 contains U2OS cells stably expressing human Neurotensin receptor type 1 (NTSR1) with no tag.

Innoprot's HiTseeker NTSR1 cell line has been designed to assay compounds or analyze their capability to modulate Neurotensin receptor type 1. When the agonist binds to NTSR1 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 NTSR1 activation process in High Throughput Screening.

### **About NTSR1**

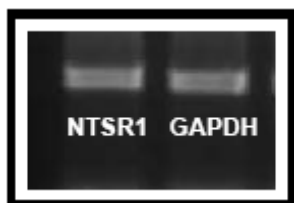
Neurotensin receptor 1 belongs to the large superfamily of G-protein coupled receptors.

NTSR1 mediates the multiple functions of neurotensin, such as hypotension, hyperglycemia, hypothermia, antinociception, and regulation of intestinal motility and secretion.

Neurotensin receptor 1 is a good target in the studies against cancer because it is thought to be implicated in several types of tumours such as breast or prostate cancers.

## **Assay Characterization**

Our expression plasmid contains the coding sequence of human NTSR1 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).



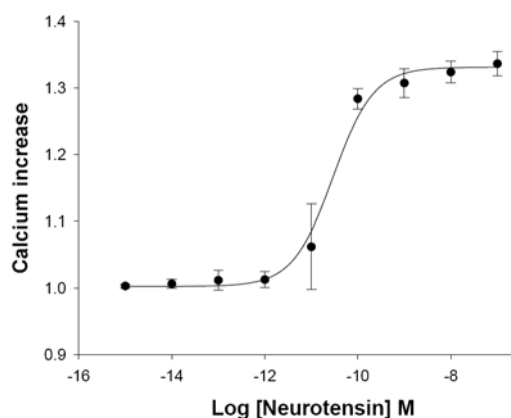
**Fig.1.** NTSR1 and GAPDH housekeeping gene RT-PCR.

## **Validation of NTSR1 cell line**

### **Calcium assay ( $EC_{50} = 2.94 \times 10^{-11} M$ )**

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 Neurotensin concentrations.



**Fig.2. NTSR1 dose response in calcium assay.**

Cells were treated with **Neurotensin** concentrations ranging from 0 to 10  $\mu M$ ,  $n=5$ . The  $EC_{50}$  for **Neurotensin** was  $\sim 2.94 \times 10^{-11} M$ . The calcium assay was validated with a  $Z' = 0.80 \pm 0.02$  for High Throughput Screening.