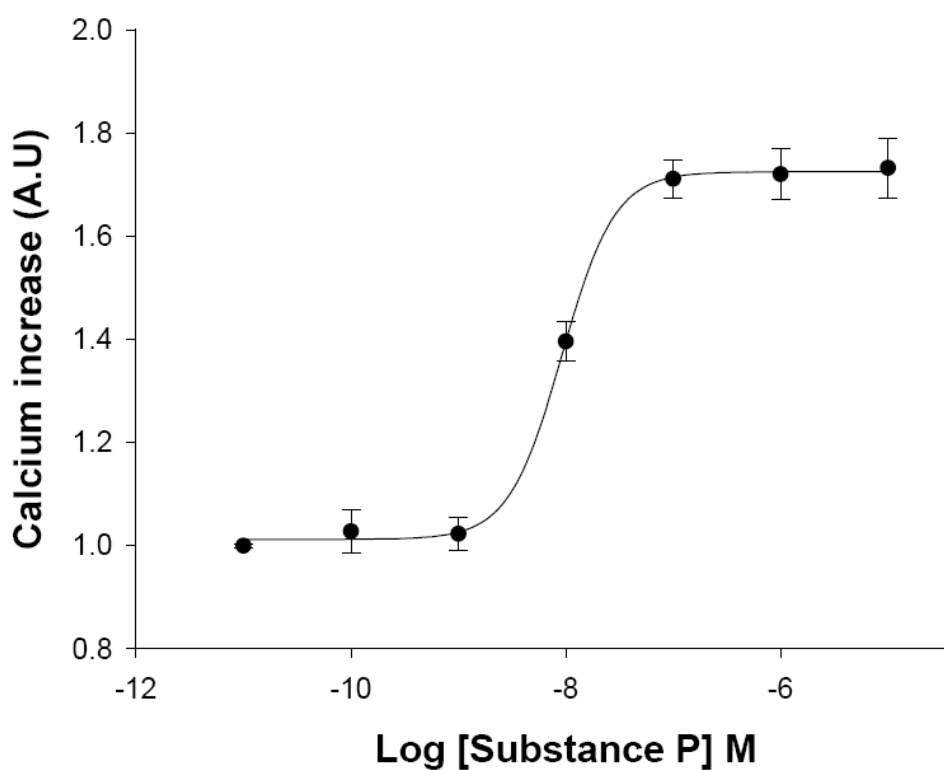


## HiTSeeker CELL LINES (LABEL-FREE GPCRS)

### - TACHYKININ RECEPTOR 2 CELL LINE -



**Product name:** TACR2 (NK2) /U2OS cell line

**EC<sub>50</sub> Substance P:**  $9.1 \times 10^{-9}$  M

**Z':** 0.75 $\pm$  0.02

## - TACHYKININ RECEPTOR 2 U2OS CELL LINE -

<b>Product Name:</b>	TACR2 (NK2)/U2OS
<b>Official Full Name:</b>	Tachykinin receptor 2
<b>DNA Accesion Number:</b>	GenBank: AY322545
<b>Host Cell:</b>	U2OS
<b>Format:</b>	Cryopreserved vials
<b>Resistance:</b>	G418
<b>Size:</b>	<i>P30148</i> : 2 vials of $3 \times 10^6$ proliferative cells <i>P30148-DA</i> : 1 vial of $2.5 \times 10^6$ division-arrested cells
<b>Storage:</b>	Liquid Nitrogen

### **Assay Briefly description**

Each vial of HiTSeeker TACR2 contains U2OS cells stably expressing human Tachykinin receptor 2 (TACR2) with no tag.

HiTSeeker TACR2 cell line has been designed to assay compounds or analyze their capability to modulate Tachykinin receptor 2. When the agonist binds to TACR2 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 TACR2 activation process in High Throughput Screening.

### **About TACR2**

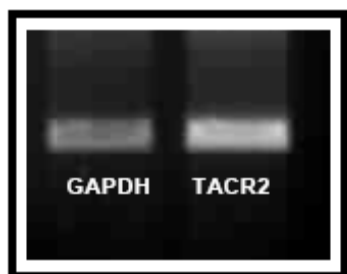
Tachykinin receptor 2 is the gene that encodes a protein that is one of the three Tachykinin receptors (TACRs), also termed NKRs.

The Tachykinin receptor family is a group of G-coupled receptors whose principal ligands are the Neurokinins.

TACR2 has been involved in stress induced hippocampal acetylcholine release and it is thought to be related with Alzheimer's disease (AD).

## **Assay Characterization**

Our expression plasmid contains the coding sequence of human TACR2 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).



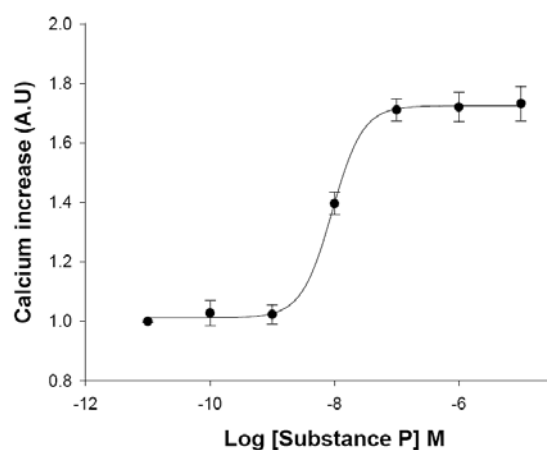
**Fig.1. GAPDH housekeeping gene and TACR2 RT-PCR.**

## **Validation of TACR2 cell line**

### **Calcium assay ( $EC_{50} = 9.1 \times 10^{-9}M$ )**

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 Substance P concentrations.



**Fig.2. TACR2 dose response in calcium assay.**

Cells were treated with **Substance P** concentrations ranging from 0 to 10  $\mu M$ ,  $n=5$ . The  $EC_{50}$  for **Substance P** was  $\sim 9.1 \times 10^{-9}M$ . The calcium assay was validated with a  $Z' = 0.75 \pm 0.02$  for High Content Screening.