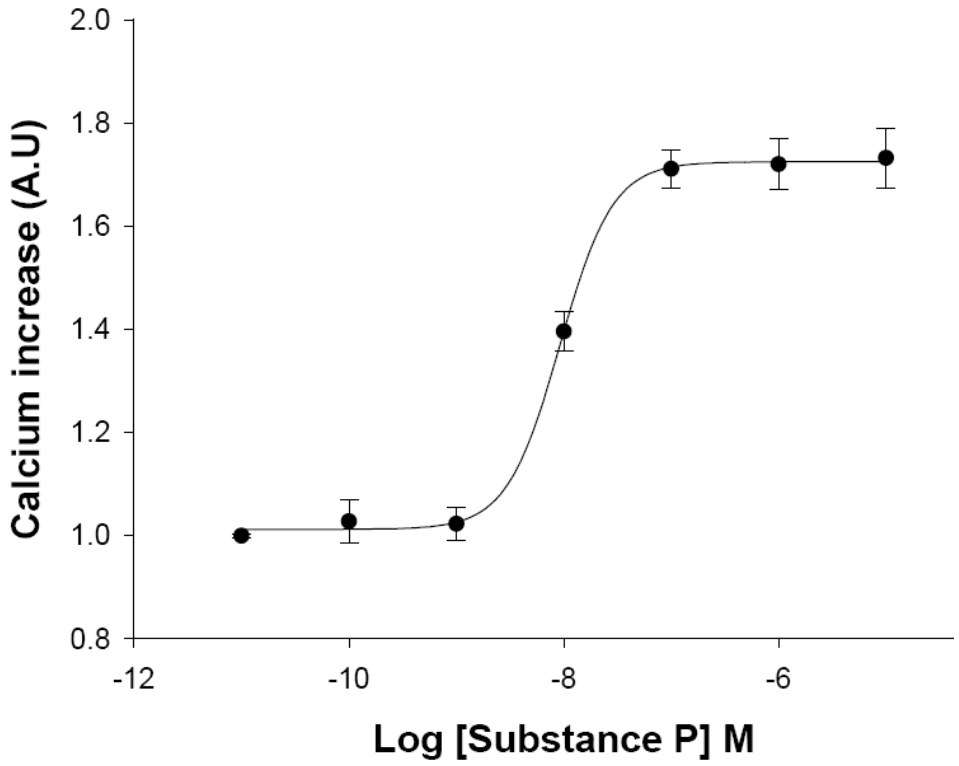
REF: P30148

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- TACHYKININ RECEPTOR 2 CELL LINE -



Product name: TACR2 (NK2) /U2OS cell line

Ec_{50} Substance P: 9.1x 10^{-9} M

Z´: 0.75+/- 0.02

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

REF: P30148

- TACHYKININ RECEPTOR 2 U2OS CELL LINE -

Product Name:	TACR2 (NK2)/U2OS
Official Full Name:	Tachykinin receptor 2
DNA Accesion Number:	GenBank: AY322545
Host Cell:	U2OS
Format:	Cryopreserved vials
Resistance:	G418
Size:	*P30148*: 2 vials of 3 x 10^6 proliferative cells
	P30148-DA: 1 vial of 2.5x10^6 division-arrested cells
Storage:	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).

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

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).

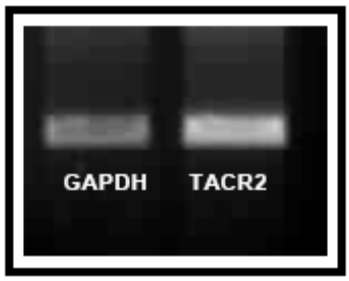


Fig.1. GAPDH housekeeping gene and TACR2 RT-PCR.

Validation of TACR2 *cell line*

Calcium assay (Ec50 = 9.1 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 Substance P concentrations.

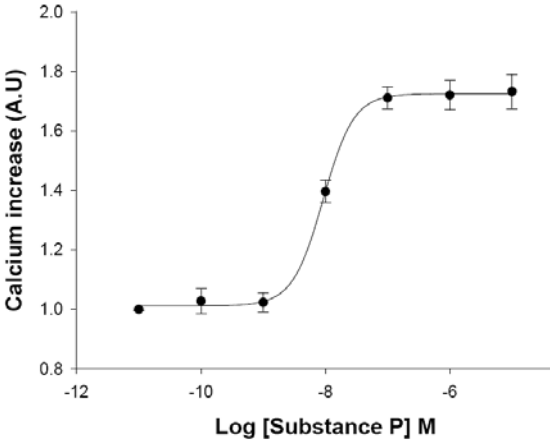


Fig.2. TACR2 **dose response in calcium assay.** Cells were treated with **Substance P** concentrations ranging from 0 to 10 µM, n=5. The EC50 for **Substance P** was ~**9.1x10^{-9}M**. The calcium assay was validated with a Z´= 0.75+/- 0.02 for High Content Screening.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com