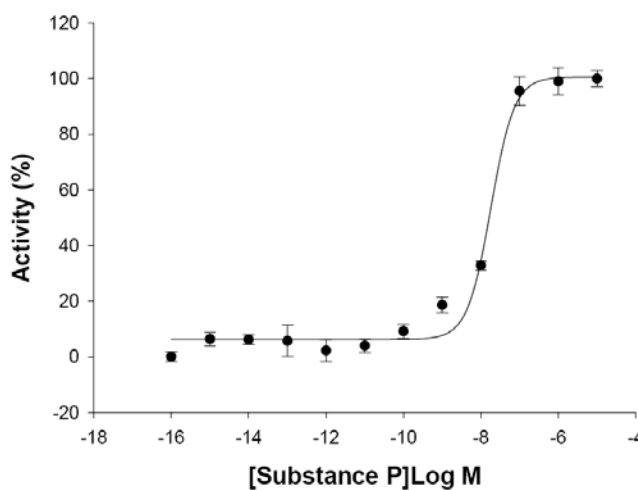
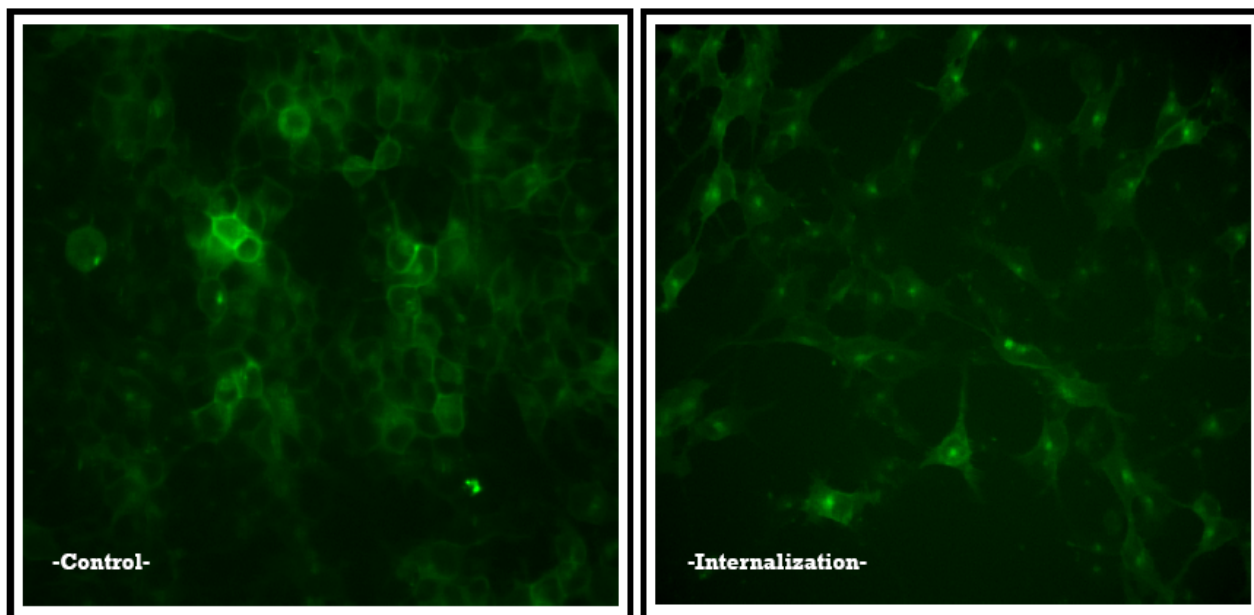


RECEPTOR INTERNALIZATION ASSAYS

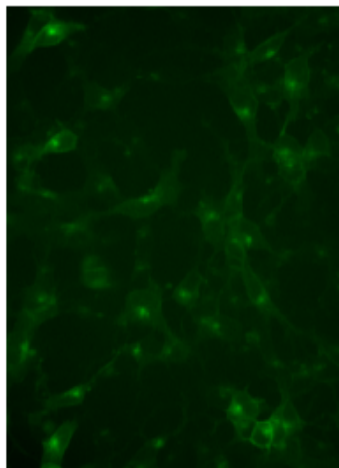
- FLUORESCENT HUMAN NEUROKININ 1 RECEPTOR CELL LINE -



Product name: NK1R-tGFP / SH-SY5Y cell line

EC₅₀ Substance P: 1.8×10^{-8} M

Z': 0.69+/- 0.02



Product Name: NK1R-tGFP/SH-SY5Y

Receptor Official Full Name: Human Neurokinin 1 Receptor (NK1R) or Tachykinin receptor 1 (TACR1)

DNA Accesion Number: GenBank: AY462098

Resistance: G418

References:

🧪 **P30229:** 2 vials of 3×10^6 proliferative cells

🧪 **P30229-DA:** 1 vial of 2×10^6 division-arrested cells

Storage: Liquid Nitrogen

🧪 **Assay Briefly description**

Each vial of NK1R-tGFP/SH-SY5Y contains SH-SY5Y cells stably expressing Human Neurokinin 1 Receptor (NK1R) tagged in the N-terminus with tGFP.

Innoprot NK1R redistribution Assay kit has been designed to assay compounds or analyze stimuli for their ability to modulate Neurokinin 1 Receptor activation and the following redistribution process inside the cells.

This highly reproducible assay allows monitoring NK1R activation and redistribution process in High Content Analysis and fluorescence microscope applications.

🧪 **About NK1R Receptor**

Neurokinin 1 receptor gene encodes the receptor for the tachykinin substance P, also referred to as Neurokinin 1.

Neurokinin receptors family is a group of G-coupled proteins whose principal ligands are Neurokinins.

The NK1 receptor is localized in high concentrations in the CNS and it is thought to mediate central stress reactions, mood control, excitatory neurotransmission, etc.

The binding of SP (Substance P) to the receptor seems to be implicated in the transmission of stress signals and pain.

Assay Characterization

Our expression plasmid containing the coding sequence of Human Neurokinin 1 Receptor (NK1R) tagged in the N-terminal with tGFP protein. Our plasmid was transfected in SH-SY5Y cells. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).

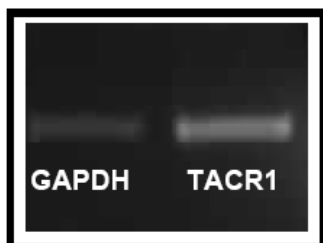


Fig.1. GAPDH housekeeping gene and TACR1 RT-PCR.

Activation and Internalization assay for TACR1-tGFP ($E_{c50} = 1.8 \times 10^{-8}M$)

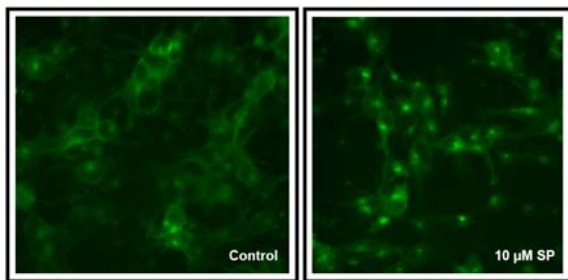


Fig.2. Internalization of TACR1 stimulated with Substance P. Concentrations from 0 to 10 μM were tested for 3h. Activation and internalization processes were detected and analyzed using “BD Pathway 855” High-Content Bioimager from BD Biosciences.

Assay Details

SH-SY5Y cells, stably expressing Human Neurokinin 1 Receptor (NK1R) tagged in the N-terminus with tGFP, were stimulated with increasing concentrations of Substance P agonist during 3 hours. After the treatment the fluorescent protein was internalized in vesicles in the cytosol, especially a big vesicle appeared next to the nucleus. Nuclei were stained with DAPI and Neurokinin 1 receptor fluorescence redistribution was determined measuring the generation of the vesicle using image analysis algorithms.

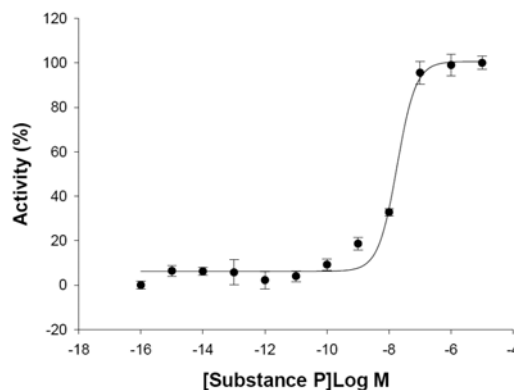


Fig.3. Concentration response curve for Substance P in Neurokinin 1 receptor cell line. Cells were treated with 12 log dilution series (n=8). The E_{c50} for the Substance P was $\sim 1.8 \cdot 10^{-8}M$ after a treatment of 3 h with agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (10 μM). The internalization assay was validated with an average of $Z' = 0.69 \pm 0.02$ for High Content Screening.