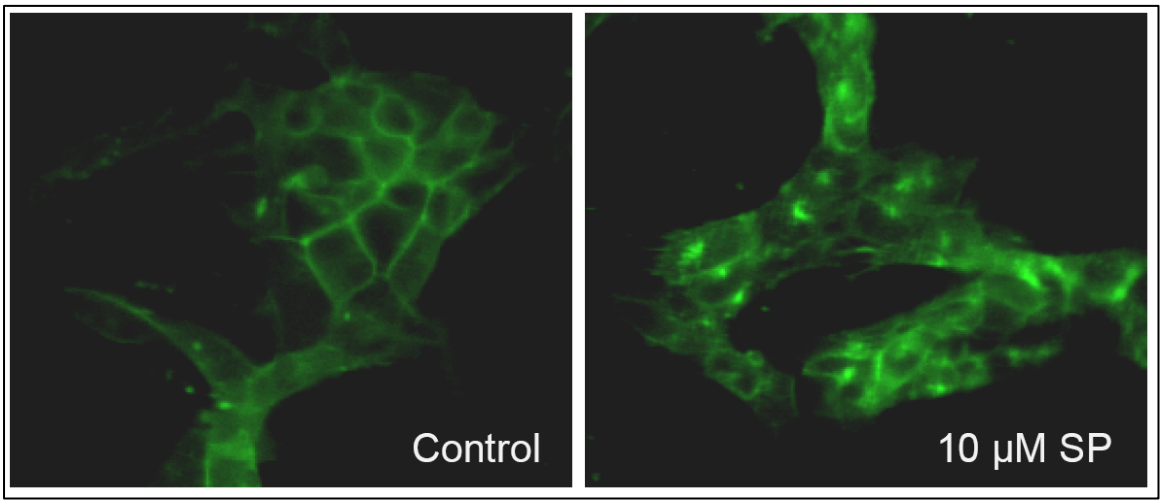
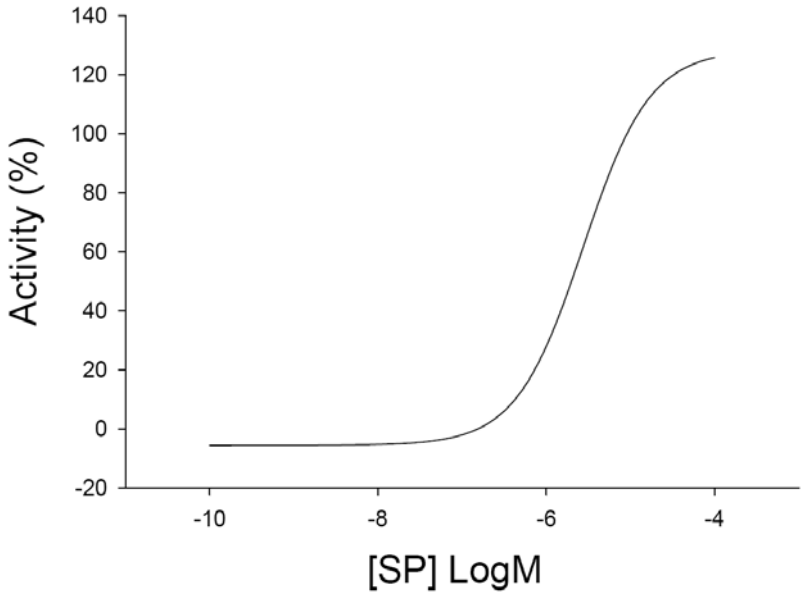
REF: P30230

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT HUMAN NEUROKININ 3 RECEPTOR CELL LINE -





Product name: NK_3R-tGFP / SH-SY5Y cell line

Ec_{50} Substance P: 2.6 x 10^{-6} M

Z´: 0.70+/- 0.1

VAT No. ESB95481909

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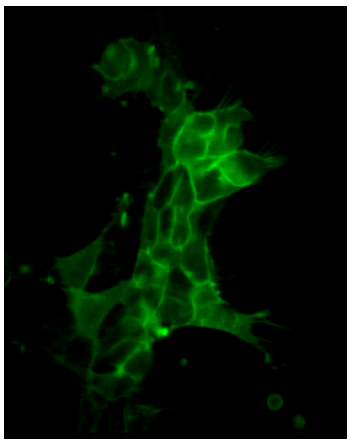


Product Name: NK_3R-tGFP/SH-SY5Y

Receptor Official Name: Human Neurokinin 3 Receptor or Tachykinin receptor 3 (TACR3)

DNA Accesion Number: NM_001059

Host Cell: SHSY5Y

Format: Cryopreserved vials

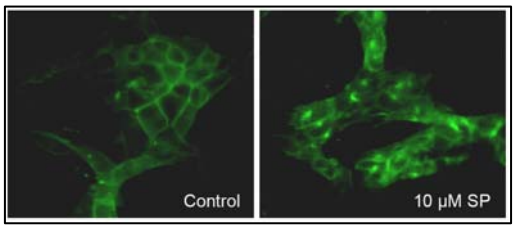
References:

- ***P30230:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30230-DA***: 1 vial of 2 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

NK_3R -tGFP/SHSY5Y contains SHSY5Y cells stably expressing human Neurokinin 3 receptor tagged in the C-terminus with tGFP. Innoprot NK_3R internalization Assay kit has been designed to assay compounds or analyze stimuli for their ability to modulate Neurokinin 3 receptor activation and the following internalization process quantifying the fluorescence distribution inside the cells.



This highly reproducible assay allows monitoring NK_3R receptor activation and redistribution process in High Content Analysis and fluorescence microscope applications.

Background

Neurokinin 3 receptor is the gene that encodes a protein that is one of the three Neurokinin receptors (NKRs), also termed as TACRs. The **Neurokinin receptor** family is a group of G-coupled receptors whose principal ligands are the Neurokinins. This protein family encompasses a wide range of functions including various parachine, autocrine and endocrine processes.

NK3 receptors are distributed widely throughout the CNS, and are found in high levels in the cerebral cortex, basal ganglia and dorsal horn of the spinal chord. They have a limited distribution in peripheral tissues, and are found in ganglia (e.g., myenteric plexus), kidney, and in a limited number of smooth muscles (e.g., rat portal vein). NK_3 antagonist has been proposed as antipsychotics.

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Assay Characterization

Our expression plasmid containing the coding sequence of human Neurokinin3 receptor tagged in the C-terminal with tGFP protein. Our plasmid was transfected in SHSY5Y cells, using calcium phosphate method. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).

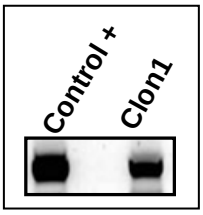

Fig.1. Clones NK3R mRNA expression.

Activation and Internalization assay for NK_3R-tGFP

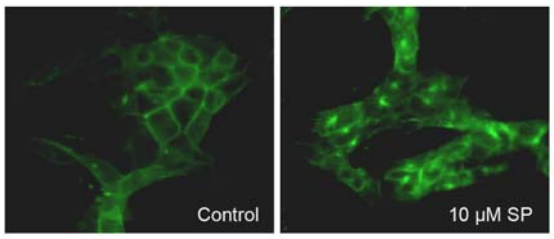

Fig.2. Internalization of NK_3R-tGFP stimulated with Substance P (SP). Cells were treated with 10uM SP for 6h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

SHSY5Y stably expressing human Neurokinin 3 receptor tagged in the C-terminus with tGFP were stimulated with different concentrations of SP agonist during 6 hours. After treatment the fluorescent protein was internalized in vesicles in the cytosol. Nuclei were stained with DAPI and NK_3R redistribution was determined as granularity in the cytosol using image analysis algorithms.

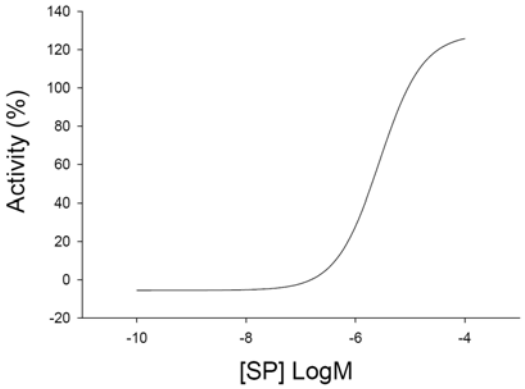

Fig.3. NK_3R-tGFP internalization in response to SP concentrations. Cells were treated with 6 log dilution series (n=8). The Ec50 for the SP was 2.6 µM after a treatment of 6h with agonist (Substance P). Cells were fixed and nuclei were stained with DAPI. % Activity was calculated relative to positive (10uM). The internalization assay was validated with an average of Z´=0.7 +/- 0.1 for High Content Screening.

Quality Controls

All cells are performance assayed and test negative for mycoplasma, bacteria, yeast and fungi. Cell viability, morphology and proliferative capacity are measured after recovery from cryopreservation. Innoprot guarantees stable expression for many generations and provides support for cell culture and visualization.

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