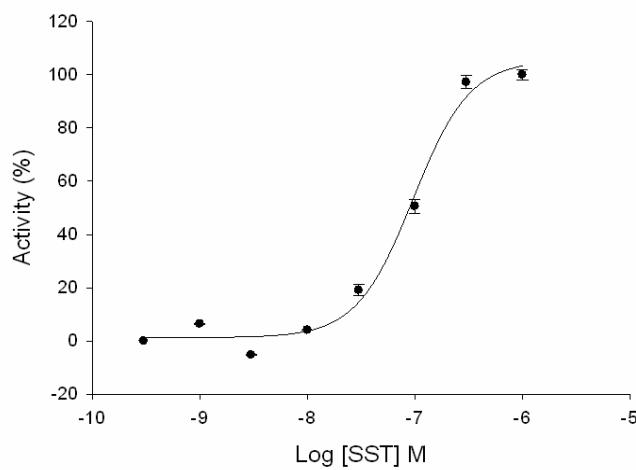
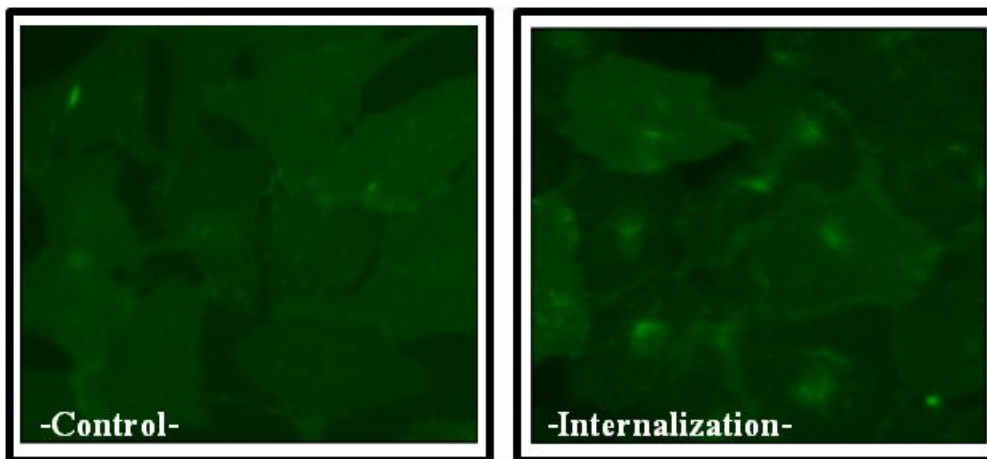


RECEPTOR INTERNALIZATION ASSAYS

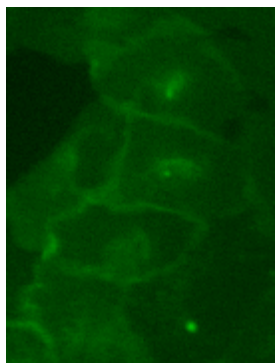
- FLUORESCENT HUMAN SOMATOSTATIN RECEPTOR TYPE 2 (SSTR2) CELL LINE -



Product name: SSTR2-tGFP / U2OS cell line

Ec₅₀ Somatostatin 28: 9.65×10^{-8} M

Z': 0.53+/- 0.02



Product Name: SSTR2-tGFP_U2OS
Reference: P30265
Rep. Official Full Name: Somatostatin receptor type 2
DNA Accession Number: Gene Bank AY236542
Host Cell: U2OS
Resistance: Puromycin
Quantity: > 3 x 10⁶ cells / vial
Storage: Liquid Nitrogen

Assay Briefly description

Each vial of SSTR2 Internalization Cell Line contains U2OS cells stably expressing human Somatostatin receptor type 2 tagged in the N-terminus with tGFP protein.

Innoprot SSTR2 Internalization assay cell line has been designed to assay potential agonists/antagonists against SSTR2, modulating its activation and the following redistribution process inside the cells. This cell line will allow the image analysis of the stimuli induced by the compounds.

This highly reproducible assay has been validated using Somatostatin 28 as a SSTR2 agonist in a High Content Analysis (HCA).

About Somatostatin receptor type 3

The diverse biological effects of somatostatin (SST) are mediated through a family of G protein coupled receptors with 5 members (SSTR1-SSTR5).

Somatostatin receptor family is increasingly interesting due to the success of its *"in vivo"* targeting and because they have been involved in Alzheimer disease.

SSTR2 is a member of the superfamily of receptors having seven transmembrane segments and is expressed in highest levels in cerebrum and kidney.

Assay Characterization

Our expression plasmid containing the coding sequence of human Somatostatin receptor type 2 tagged in the N-terminal with tGFP protein. Our plasmid was transfected in U2OS cells. Resistant clones were obtained by limit dilution, and receptor gene expression was tested by RT-PCR (Fig.1).

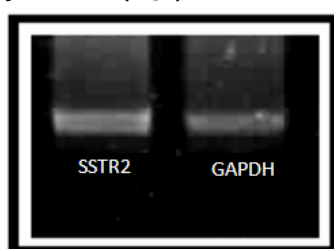


Fig1. SSTR2 and GAPDH housekeeping gene RT-PCR.

Activation and Internalization assay for SSTR2-tGFP ($E_{c50} = 9.65 \times 10^{-8} M$)

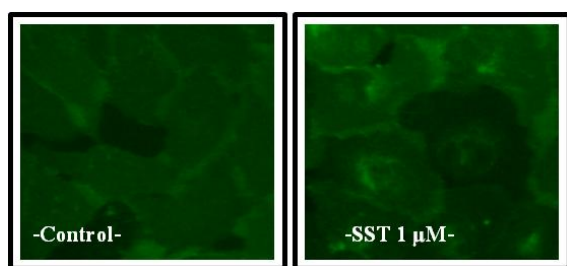


Fig2. Internalization of SSTR2 stimulated with human Somatostatin 28. Concentrations from 0 to 1 μM were tested for 1.5h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

U2OS cells, stably expressing human Somatostatin receptor type 2 tagged in the N-terminus with tGFP protein, were stimulated with increasing concentrations of **human Somatostatin 28 during 1.5 h**. After the treatment an accumulation of fluorescence was observed around nucleus. Nuclei were stained with DAPI and SSTR2 fluorescence redistribution was determined measuring the increase of fluorescence surrounding the nuclei using image analysis algorithms.

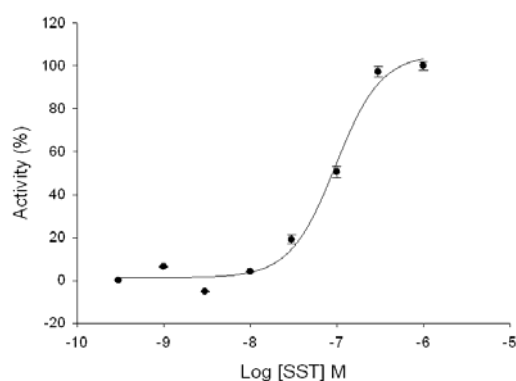


Fig3. Concentration response curve for human Somatostatin 28 in SSTR2 cell line. Cells were treated with 8 log dilution series (n=5). The E_{c50} for human Somatostatin 28 was $\sim 9.65 \times 10^{-8} M$ after a treatment of 1.5 h with the agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (1μM). The internalization assay was validated with an average of $Z' = 0.53 \pm 0.02$ for High Content Screening.