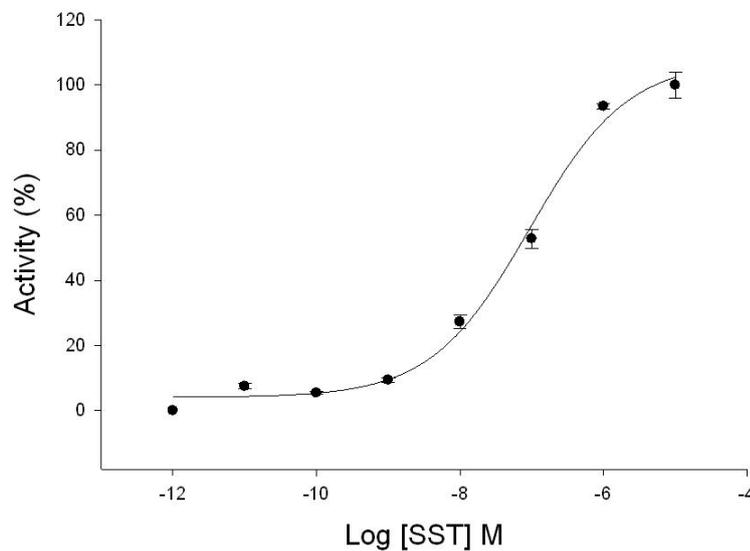
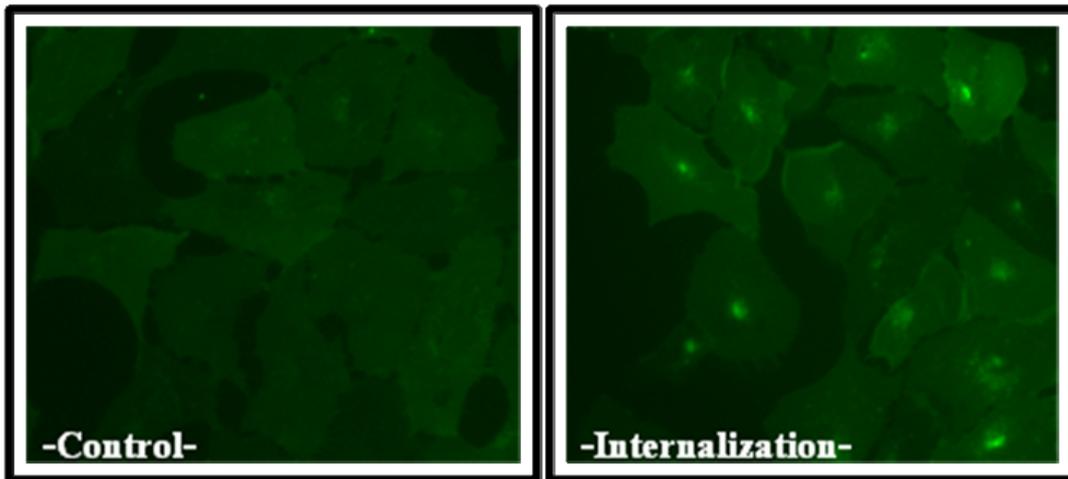


## RECEPTOR INTERNALIZATION ASSAYS

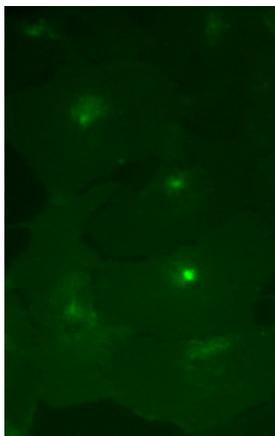
- FLUORESCENT HUMAN SOMATOSTATIN RECEPTOR TYPE 3 (SSTR3) CELL LINE -



**Product name:** SSTR3-tGFP (SS3R-tGFP) / U2OS cell line

**Ec<sub>50</sub> Somatostatin 28:**  $9.25 \times 10^{-8}$  M

**Z':** 0.66+/- 0.02



**Product Name:** SSTR3-tGFP\_U2OS

**Reference:** P30266

**Rep. Official Full Name:** Somatostatin receptor type 3

**DNA Accession Number:** Gene Bank AY322541

**Host Cell:** U2OS

**Resistance:** Puromycin

**Quantity:** > 3 x 10<sup>6</sup> cells / vial

**Storage:** Liquid Nitrogen

### **Assay Briefly description**

Each vial of Innoprot SSTR3 Internalization Assay cell line contains U2OS cells stably expressing human Somatostatin receptor type 3 tagged in the N-terminus with tGFP protein.

Innoprot SSTR3 internalization cell line has been designed to assay potential agonists/antagonists against SSTR3, 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 SSTR3 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.

SSTR3 is a member of the superfamily of receptors having seven transmembrane segments and is expressed in highest levels in brain and pancreatic islets. SSTR3 is functionally coupled to adenylyl cyclase.

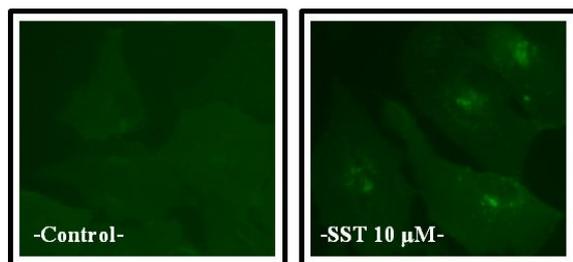
### **Assay Characterization**

Our expression plasmid containing the coding sequence of human Somatostatin receptor type 3 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. SSTR3 and GAPDH** housekeeping gene RT-PCR.

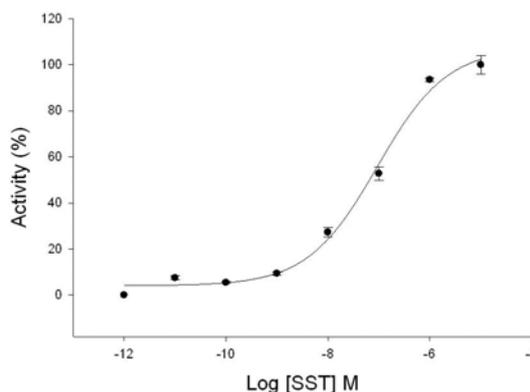
### **Activation and Internalization assay for SSTR3-tGFP ( $E_{c50} = 9.25 \times 10^{-8} M$ )**



**Fig2. Internalization of SSTR3 stimulated with human Somatostatin 28.** 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**

U2OS cells, stably expressing human Somatostatin receptor type 3 tagged in the N-terminus with tGFP protein, were stimulated with increasing concentrations of **human Somatostatin 28 during 3 h.** After the treatment an accumulation of fluorescence was observed around nucleus. Nuclei were stained with DAPI and CCR3 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 SSTR3 cell line.** Cells were treated with 8 log dilution series (n=5). The  $E_{c50}$  for human Somatostatin 28 was  $\sim 9.25 \times 10^{-8} M$  after a treatment of 3 h with the agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (10μM). The internalization assay was validated with an average of  $Z' = 0.66 \pm 0.02$  for High Content Screening.