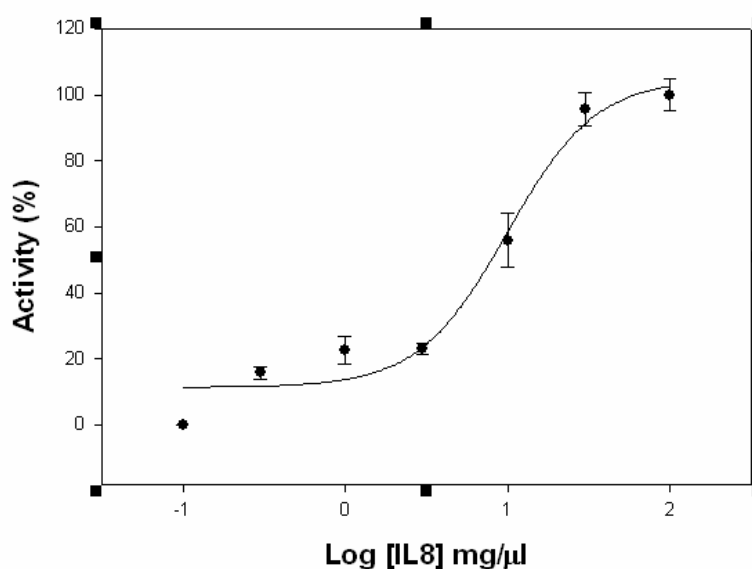
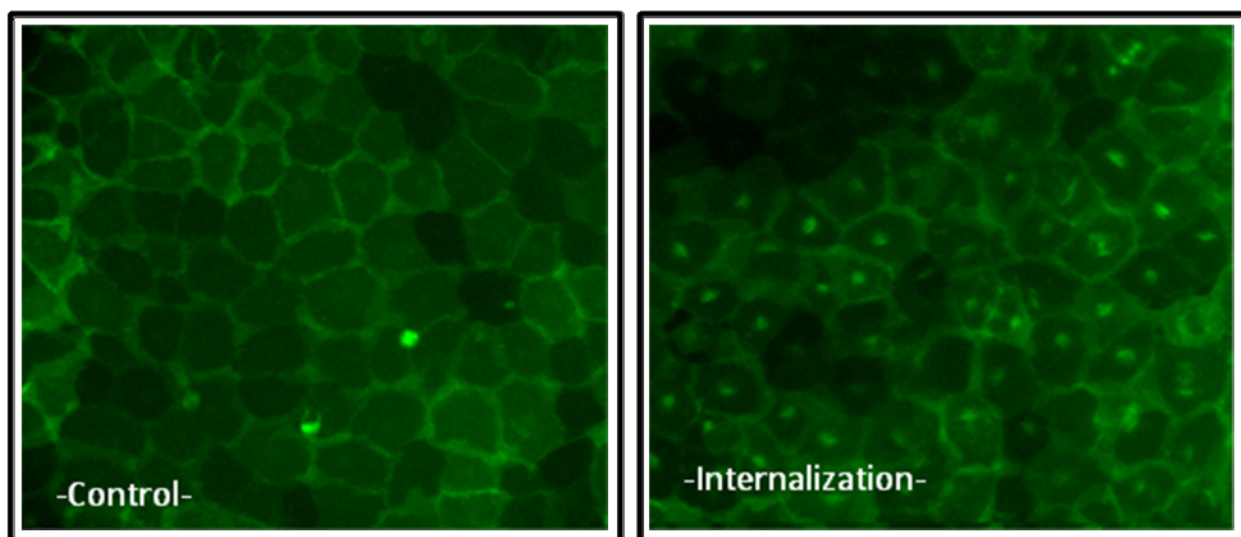


## RECEPTOR INTERNALIZATION ASSAYS

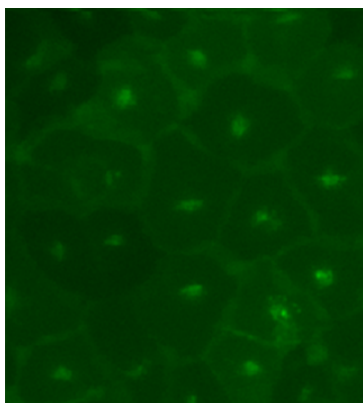
- FLUORESCENT HUMAN CXCR2 CELL LINE -



**Product name:** CXCR2-tGFP (IL8Rβ-tGFP) / U2OS cell line

**EC<sub>50</sub> IL-8:** 9.9 mg/μl

**Z':** 0.73+/- 0.02



**Product Name:** IL8R $\beta$ /CXCR2-tGFP\_U2OS

**Reference:** P30274

**Rep. Official Full Name:** chemokine (C-X-C motif) receptor 2

**DNA Accession Number:** Gene Bank M73969

**Host Cell:** U2OS

**Resistance:** Puromycin

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

**Storage:** Liquid Nitrogen

### **Assay Briefly description**

Each Vial of CXCR2 Internalization Assay Cell Line contains U2OS cells stably expressing human chemokine (C-X-C motif) receptor 2 tagged in the N-terminus with tGFP protein.

Innopro CXCR2 internalization cell line has been designed to assay potential agonists/antagonists against CXCR2, 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 **IL8** as a CXCR2 (IL8R $\beta$ ) agonist in a High Content Analysis (HCA).

### **About Interleukin 8 receptor beta**

Interleukin 8 receptor beta is a chemokine receptor.

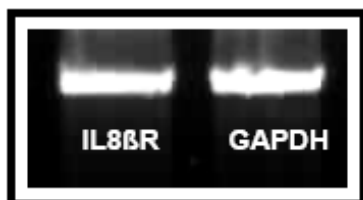
Chemokines and chemokines receptors play roles in leukocyte recruitment and cellular functions as activation, proliferation, and differentiation.

This receptor mediates neutrophil migration to sites of inflammation.

Interleukin 8 receptor beta mediates the angiogenic response of IL8 in intestinal microvascular endothelial cells.

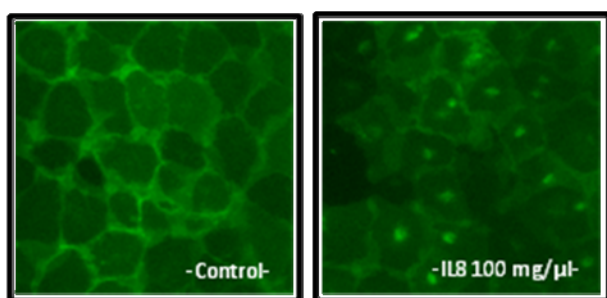
## Assay Characterization

Our expression plasmid containing the coding sequence of human chemokine (C-X-C motif) receptor 2 (Interleukin 8 receptor beta) 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. CXCR2 and GAPDH housekeeping gene RT-PCR.**

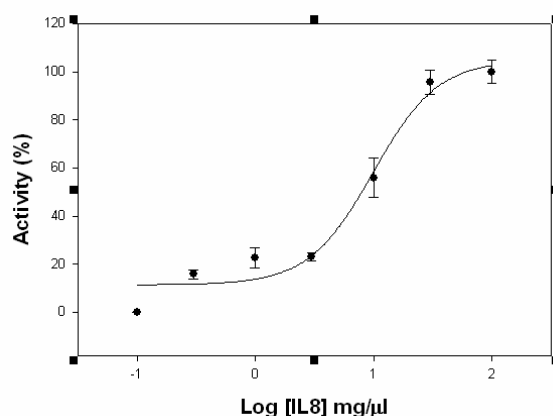
## Activation and Internalization assay for CXCR2 (IL8Rβ) -tGFP (Ec50 = 9.9 mg/μl)



**Fig2. Internalization of IL8Rβ /CXCR2 stimulated with IL8.** Concentrations from 0 to 100 mg/μl were tested for 24 h. 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 Interleukin 8 receptor beta tagged in the N-terminus with tGFP protein, were stimulated with increasing concentrations of **IL8 during 24 h**. After the treatment an accumulation of fluorescence was observed around nucleus. Nuclei were stained with DAPI and CXCR2 fluorescence redistribution was determined measuring the increase of fluorescence surrounding the nuclei using image analysis algorithms.



series (n=5). The Ec50 for IL8 was ~ 9.9 mg/μl after a treatment of 24 h with the agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (100 mg/μl). The internalization assay was validated with an average of  $Z' = 0.73 \pm 0.02$  for High Content Screening.