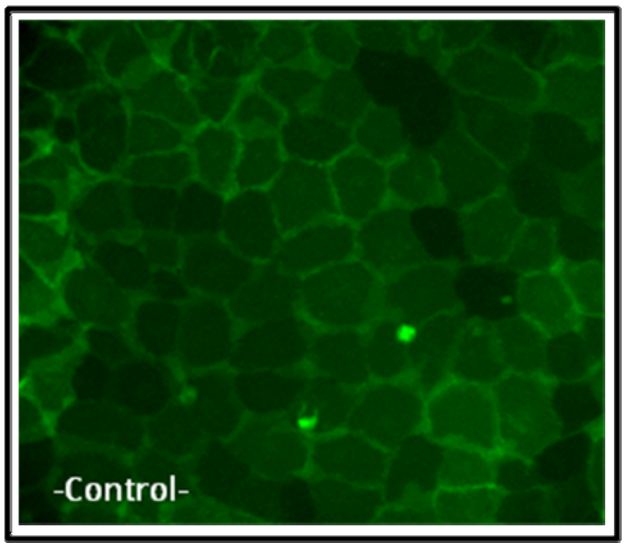
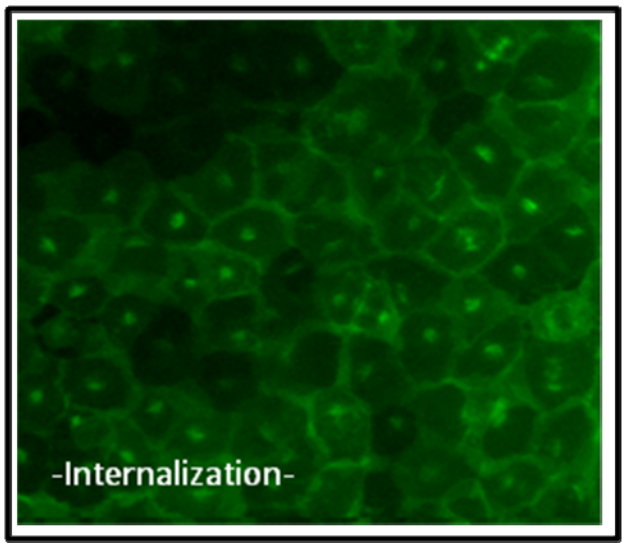
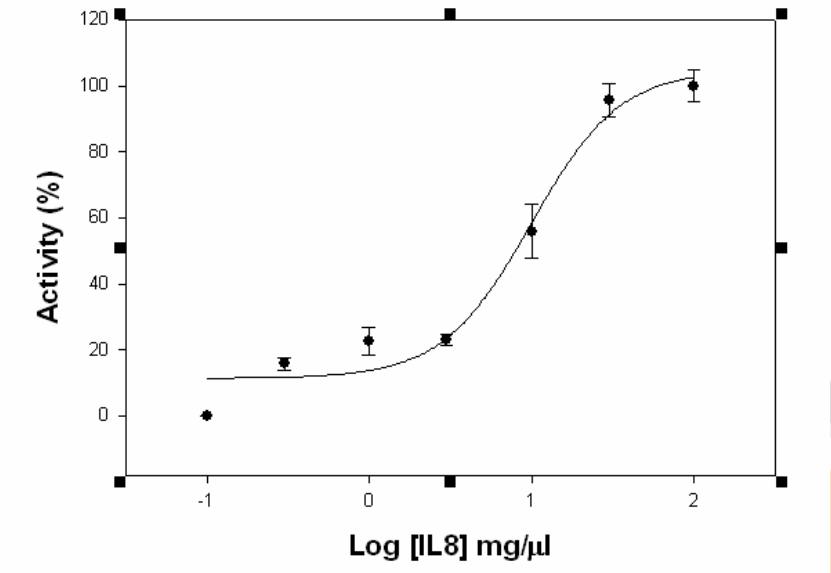
REF: P30274

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT HUMAN CXCR2 CELL LINE -







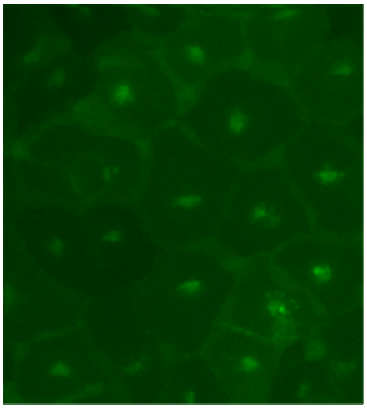
Product name: CXCR2-tGFP (IL8Rß-tGFP) / U2OS cell line

Ec_{50} IL-8: 9.9 mg/µl

Z´: 0.73+/- 0.02

VAT No. ESB95481909

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com



Product Name: IL8Rß/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 10^6 cells / vial

Storage: Liquid Nitrogen

Assay Briefly description

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

Innoprot 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 (IL8Rß) 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.

VAT No. ESB95481909

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

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).

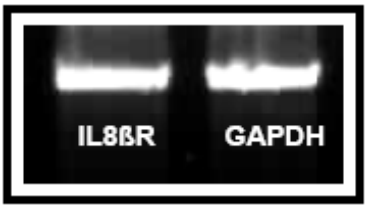


Fig1. CXCR2 and GAPDH housekeeping gene RT-PCR.

Activation and Internalization assay for CXCR2 (IL8Rß) -tGFP (Ec50 = 9.9 mg/µl)

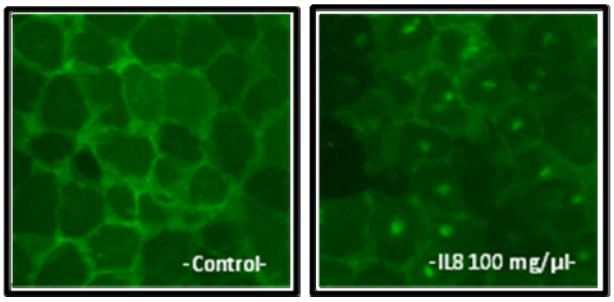
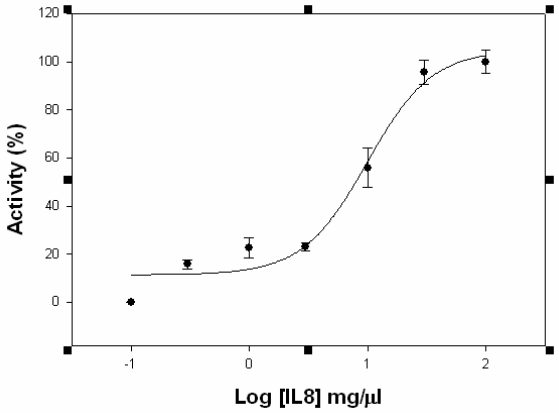


Fig2. Internalization of IL8Rß /CXCR2 stimulated with IL8. Concentrations from 0 to 100 mg/µl were tested for 24 h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

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+/- 0.02 f**or High Content Screening.

VAT No. ESB95481909
INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com