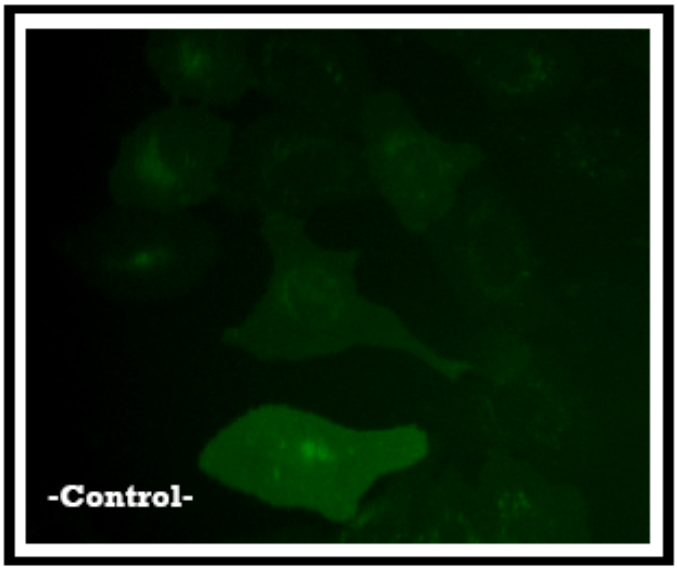
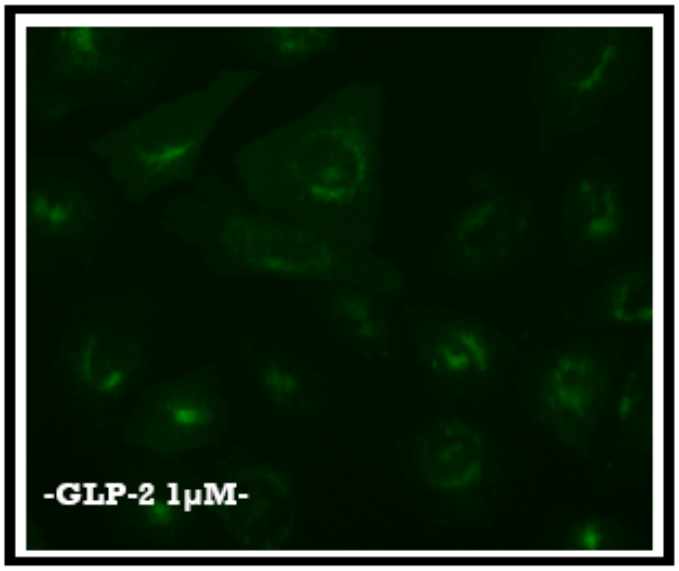
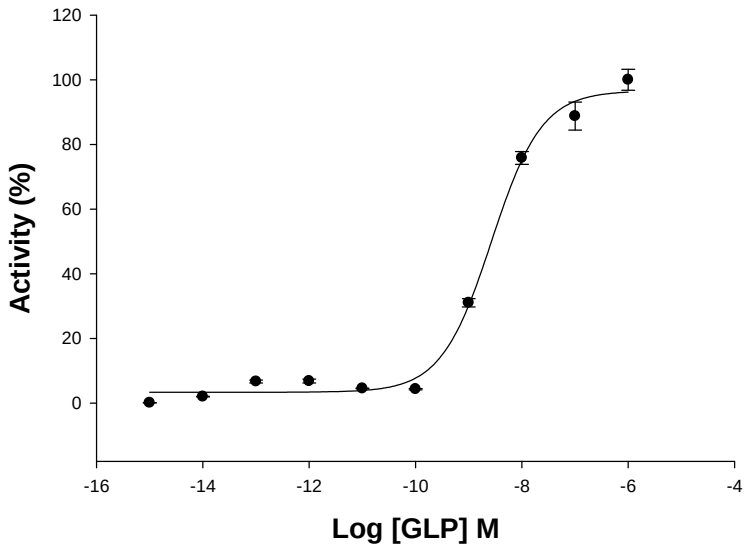
REF: P30227

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT HUMAN GLUCAGON-LIKE PEPTIDE 2 RECEPTOR CELL LINE -







Product name: GLP2R-tGFP / U2OS cell line

Ec_{50} Dopamine: 2.67 x 10^{-9} M

Z´: 0.74+/- 0.02

VAT No. ESB95481909

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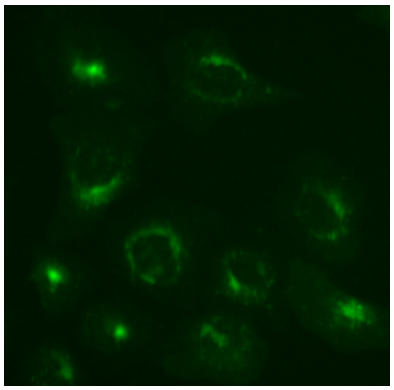

Product Name: GLP2R-tGFP_U2OS

Reference: P30227

Recp. Official Full Name: Glucagon like peptide 2 receptor

DNA Accesion Number: BC096261

Host Cell: U2OS

Resistance: G418

References:

- ***P30227:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30227-DA***: 1 vial of 2 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

GLP2R-tGFP_U2OS contains U2OS cells stably expressing human Glucagon like peptide 2 Receptor (GLP2R) tagged in the N-terminus with tGFP protein.

Innoprot GLP2R redistribution cell line has been designed to assay potential agonists/ antagonists against GLP2R, 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 GLP-2 as a GLP2R agonist in a High Content Analysis (HCA).

About Delta opioid receptor

GLP2 receptor (GLP2R) is a G protein-coupled receptor superfamily member related to glucagon receptor and GLP1 receptor.

Glucagon like peptide 2 receptor is expressed in SNC and gut.

GLP2R transduces the biological actions of GLP-2, a 33 aminoacid peptide produced by the intestinal endocrine L cell and by various neurons in the central nervous system. Intestinal GLP-2 is co-secreted along with GLP-1 upon nutrient ingestion.

GLP-2 produces several actions such as including intestinal growth, enhancement of intestinal function, reduction in bone breakdown and neuroprotection.

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Assay Characterization

Our expression plasmid containing the coding sequence of human Glucagon like peptide 2 Receptor (GLP2R) 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).

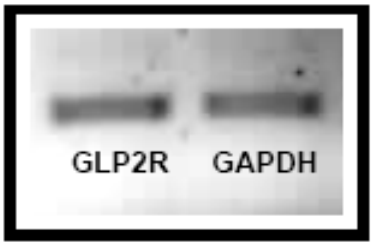

Fig1.GLP2R and GAPDH housekeeping gene RT-PCR.

Activation and Internalization assay for GLP2R-tGFP (Ec50 =2.67 x 10^{-9}M)

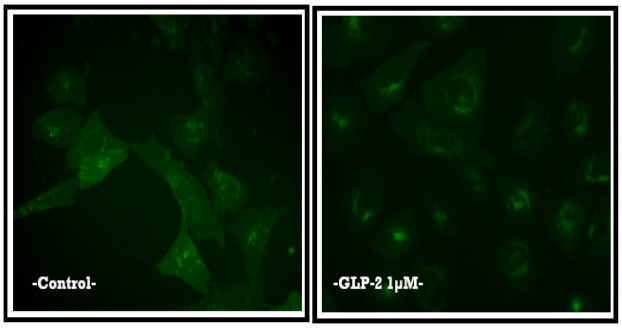

Fig2. Internalization of GLP2R stimulated with GLP-2 Concentrations from 0 to 1 µM were tested for 1h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

U2OS cells, stably expressing human Glucagon like peptide 2 receptor tagged in the N-terminus with tGFP protein, were stimulated with increasing concentrations of GLP-2 during 1h. After the treatment an accumulation of fluorescence was observed around nucleus. Nuclei were stained with DAPI and GLP2R fluorescence redistribution was determined measuring the increase of fluorescence surrounding the nuclei using image analysis algorithms.

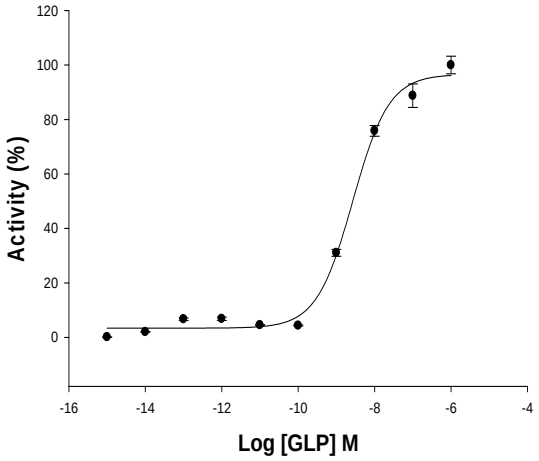

Fig3. Concentration response curve for GLP-2 in Glucagon like peptide 2 receptor cell line. Cells were treated with 10 log dilution series (n=5). The Ec50 for GLP-2 was ~ 2.67x10^{-9}M after a treatment of 1 h with the agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (1 µM). The internalization assay was validated with an average of Z´=0.74+/- 0.02 for High Content Screening.

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