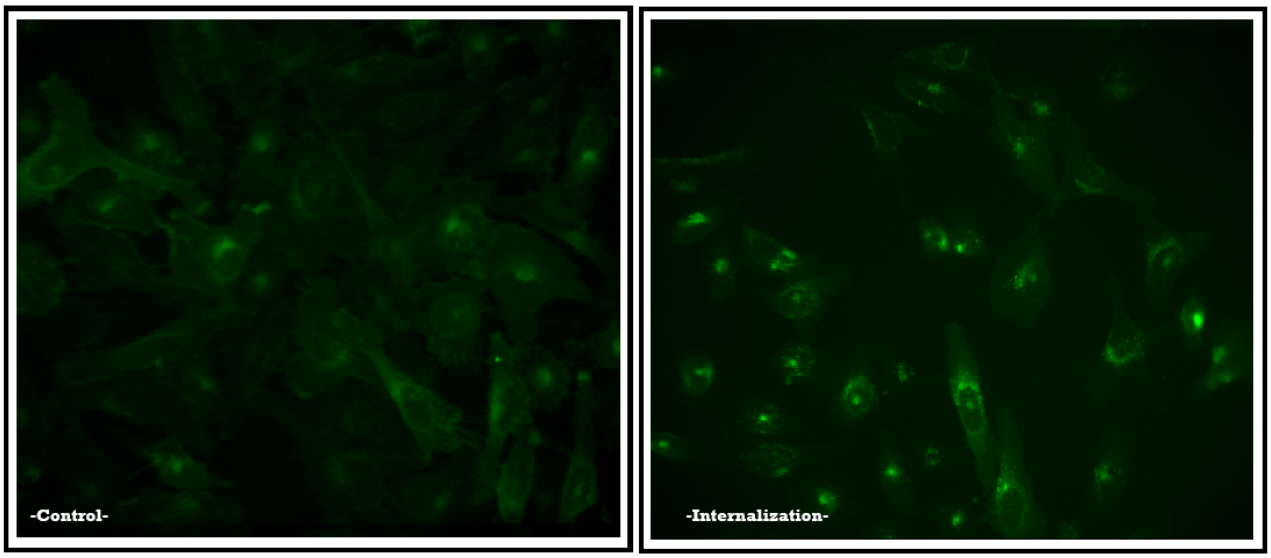
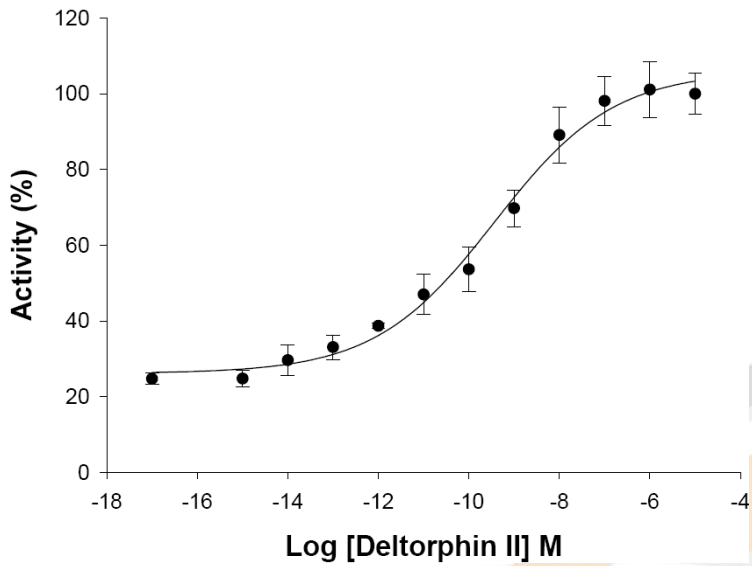
REF: P30232

RECEPTOR INTERNALIZATION ASSAYS

- FLUORESCENT HUMAN DELTA OPIOID RECEPTOR CELL LINE -





Product name: DOR-tGFP / U2OS cell line

Ec_{50} Deltorphin: 3.6 x 10^{-10} M

Z´: 0.72+/- 0.02

VAT No. ESB95481909

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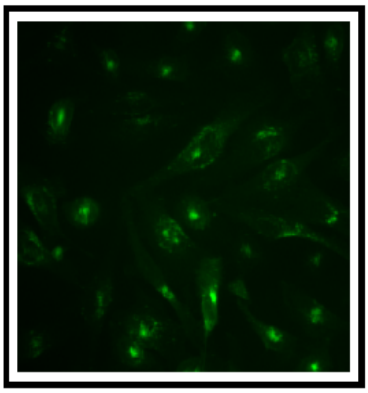

Product Name: DOR-tGFP_U2OS

Recp. Official Full Name: Delta Opioid receptor (DOR)

DNA Accesion Number: Gene Bank NM_000911

Host Cell: U2OS

Resistance: G418

References:

- ***P30232:*** 2 vials of 3 x 10^6 proliferative cells
- ***P30232-DA***: 1 vial of 2 x 10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of DOR-tGFP U2OS cell line contains 3 million U2OS cells stably expressing human Delta opioid Receptor (DOR) tagged in the N-terminus with tGFP protein.

Innoprot DOR redistribution cell line has been designed to assay potential agonists/ antagonists against DOR, 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 Deltorphin II as a DOR agonist in a High Content Analysis (HCA).

About Delta opioid receptor

The δ-opioid receptor, also known as delta opioid receptor or simply delta receptor, abbreviated DOR, is an opioid receptor that has enkephalins as its endogenous ligands.

Opioid receptors mediate the analgesic action and addictive properties of opioid drugs. The δ -receptor is a promising target for treatment of both drug addiction and mood-related disorders.

DOR is also a good target related with Alzheimer´s Disease (AD). Agonist-induced activation of the δ-opioid receptor has been shown to augment β- and γ-secretase activities, which increased the production of β-amyloid peptide (Aβ), known to accumulate in the brain tissues of Alzheimer's disease (AD) patients

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Assay Characterization

Our expression plasmid containing the coding sequence of human Delta opioid receptor 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).

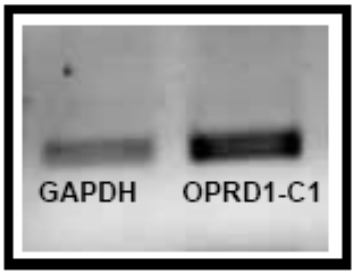

Fig1. GAPDH housekeeping gene and DOR RT-PCR.

Activation and Internalization assay for DOR-tGFP (Ec50 =3.6 x 10^{-10}M)

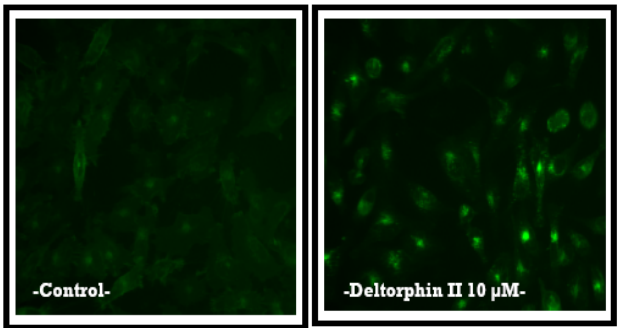

Fig2. Internalization of DOR stimulated with Deltorphin II. Concentrations from 0 to 10 µM were tested for 1h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

Assay Details

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

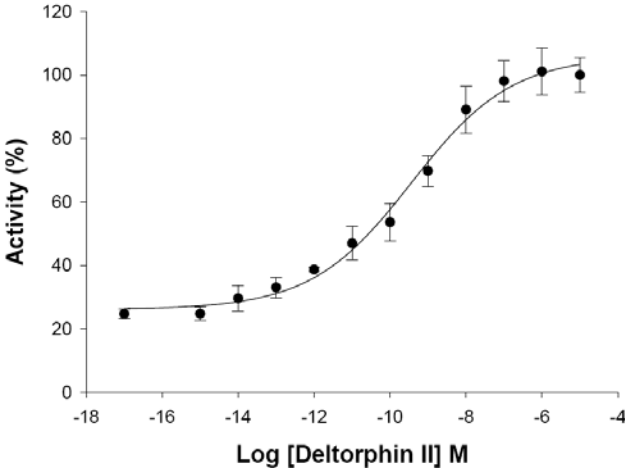

Fig3. Concentration response curve for Deltorphin II in Delta opioid receptor cell line. Cells were treated with 12 log dilution series (n=5). The Ec50 for the Deltorphin II was ~ 3.6x10^{-10}M after a treatment of 1 h with the agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (10 µM). The internalization assay was validated with an average of Z´=0.72+/- 0.02 for High Content Screening.

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