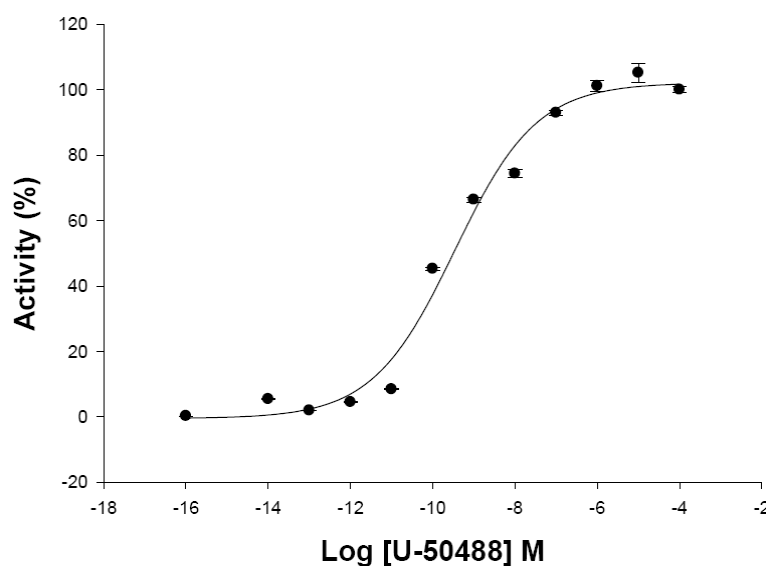
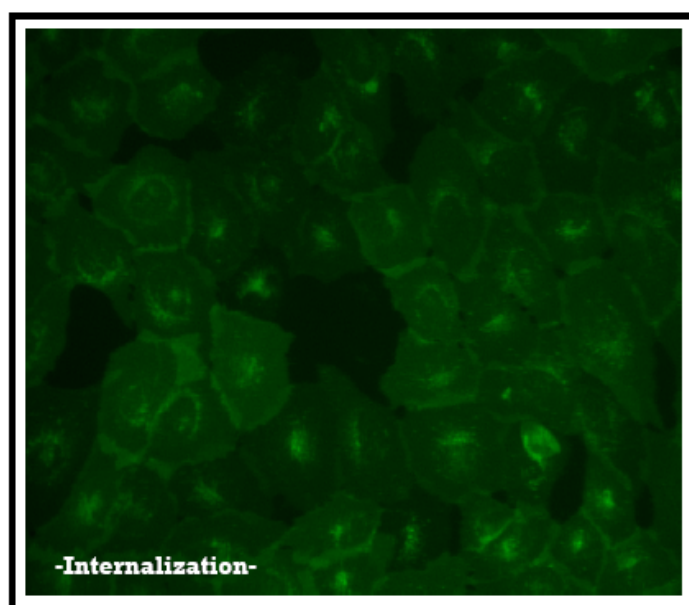
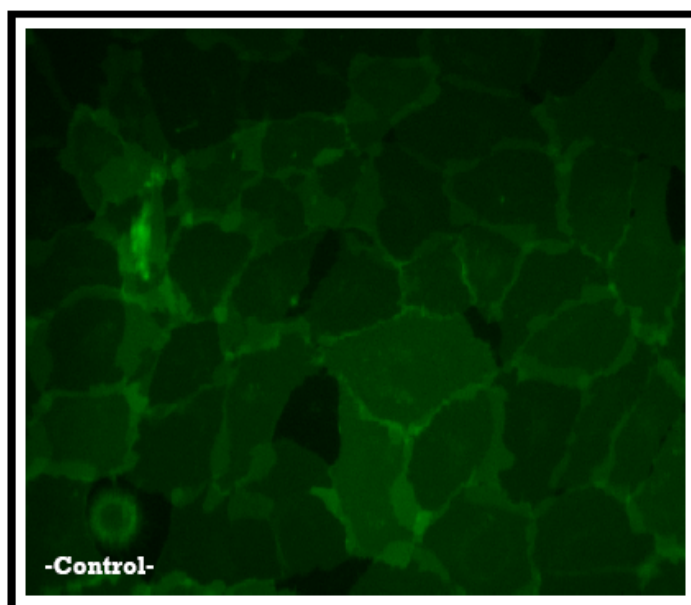


RECEPTOR INTERNALIZATION ASSAYS

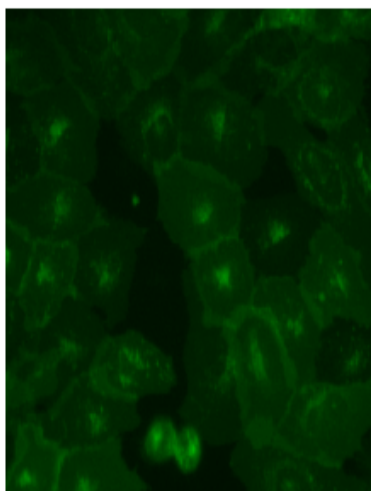
- FLUORESCENT HUMAN KAPPA OPIOID RECEPTOR CELL LINE -



Product name: KOR-tGFP / U2OS cell line

EC₅₀ U-50488: 3.42×10^{-10} M

Z': 0.84 \pm 0.02



Product Name: KOR-tGFP_U2OS

Reference: P30233


Recp. Official Full Name: Kappa Opioid receptor (KOR)


DNA Accession Number: Gene Bank U11053

Host Cell: U2OS

Resistance: G418

References:

 **P30233:** 2 vials of 3×10^6 proliferative cells

 **P30233-DA:** 1 vial of 2×10^6 division-arrested cells

Storage: Liquid Nitrogen

Assay Briefly description

Each vial of KOR-tGFP_U2OS contains U2OS cells stably expressing human Kappa opioid Receptor (KOR) tagged in the N-terminus with tGFP protein.

Innopro KOR redistribution cell line has been designed to assay potential agonists/antagonists against KOR, 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 **U-50488** as a KOR agonist in a High Content Analysis (HCA).

About Kappa opioid receptor

K-opioid receptor is one of five related receptors that bind opium-like compounds in the brain and are responsible for mediating the effects of these compounds. These effects include altering the perception of pain, consciousness, motor control, and mood.

Opioid receptors mediate the analgesic action and addictive properties of opioid drugs. The K-opioid receptor may provide a natural addiction control mechanism, and consequently selective agonists of this receptor may have therapeutic potential in the treatment of addiction.

Assay Characterization

Our expression plasmid containing the coding sequence of human Kappa 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).

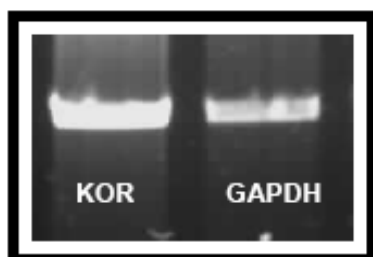


Fig1. KOR and GAPDH housekeeping gene RT-PCR.

Activation and Internalization assay for KOR-tGFP ($EC_{50} = 3.42 \times 10^{-10} M$)

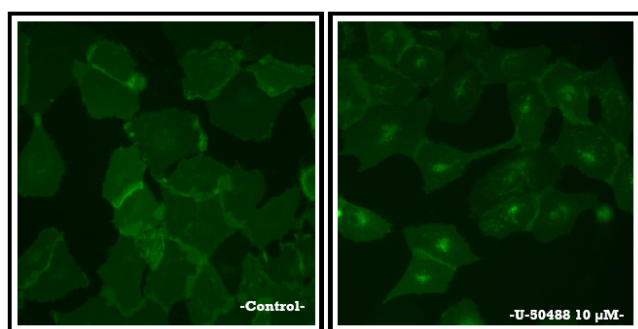


Fig2. Internalization of KOR stimulated with U-50488. Concentrations from 0 to 10 μM were tested for 2h.

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 Kappa opioid receptor tagged in the N-terminus with tGFP protein, were stimulated with increasing concentrations of **U-50488** during 2h. 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.

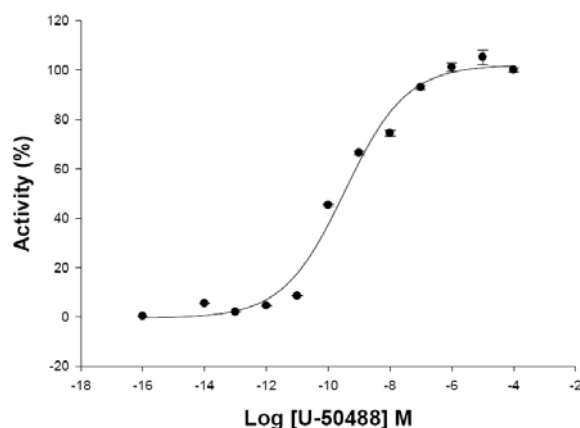


Fig3. Concentration response curve for U-50488 in Kappa opioid receptor cell line. Cells were treated with 12 log dilution series (n=5). The EC_{50} for the **U-50488** was $\sim 3.42 \times 10^{-10} M$ after a treatment of 2 h with the agonist.

Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (100 μM). The internalization assay was validated with an average of $Z' = 0.84 \pm 0.02$ for High Content Screening.