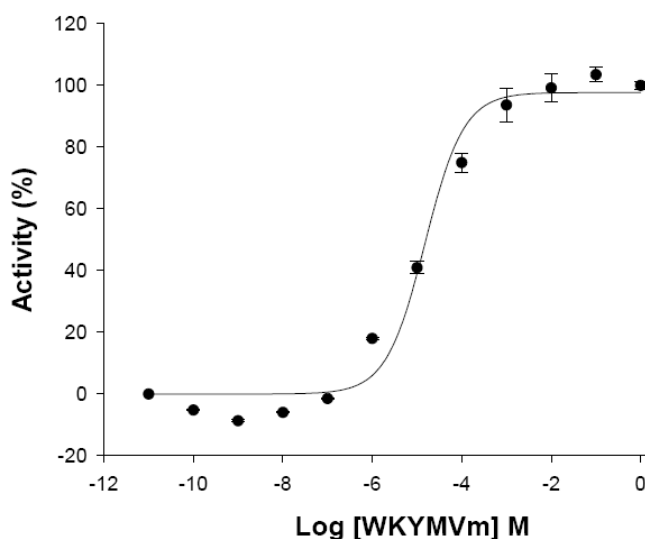
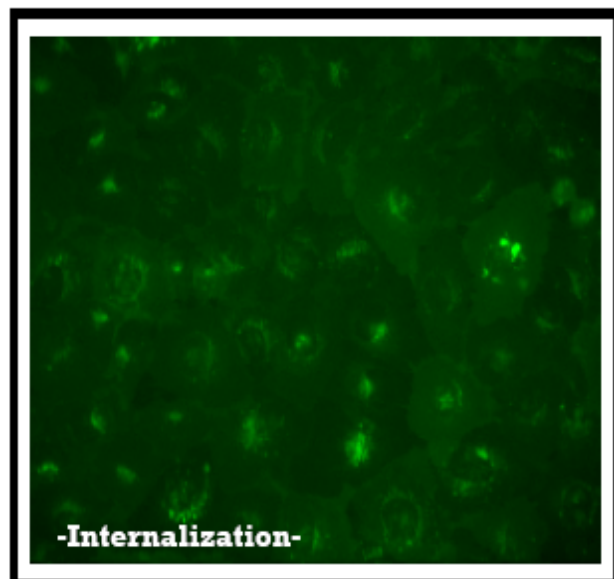
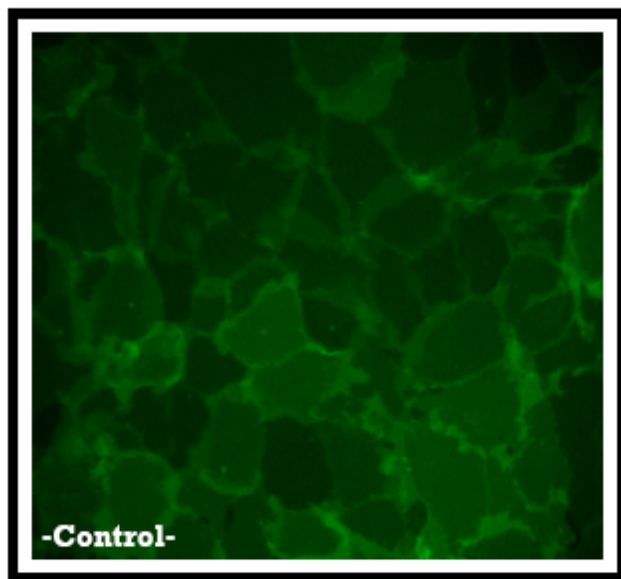


## RECEPTOR INTERNALIZATION ASSAYS

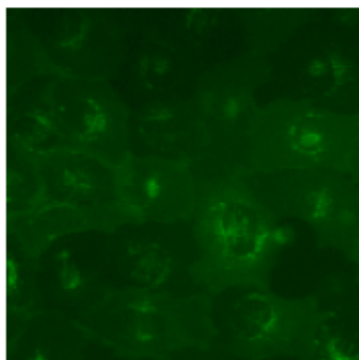
- HUMAN FORMYL PEPTIDE RECEPTOR 2 (FPR2/ALX) / (FPRL1) CELL LINE -



**Product name:** FPR2/ALX-tGFP (FPRL1-tGFP)/ U2OS cell line

**EC<sub>50</sub> WKYMVm:** 1.5x10<sup>-5</sup> µg/ml

**Z':** 0.70 +/- 0.02



**Product Name:** FPR2/ALX-tGFP\_U2OS

**Reference:** P30276

**Rep. Official Full Name:** Formyl peptide receptor 2

**DNA Accession Number:** Gene Bank AY225226

**Host Cell:** U2OS

**Resistance:** Puromycin

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

**Storage:** Liquid Nitrogen

### **Assay Briefly description**

Each vial of FPR2/ALX-tGFP U2OS Internalization Assay Cell Line contains U2OS cells stably expressing human Formyl peptide receptor 2 tagged in the N-terminus with tGFP protein.

Innoprot FPR2/ALX Internalization Assay cell line has been designed to assay potential agonists/ antagonists against FPR2/ALX, 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 **WKYMVM** as a FPR2/ALX agonist in a High Content Analysis (HCA).

### **About Formyl peptide receptor 2**

The **formyl peptide receptor: (FPR)** are a family of G protein-coupled receptors involved in chemotaxis.

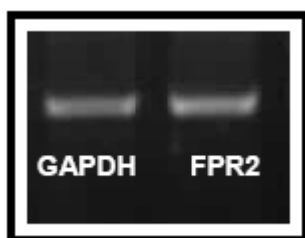
Formyl peptide receptor 1 (FPR1) and FPR2/ALX are known to control neutrophil chemotaxis in response to various ligands.

FPR2/ALX can mediate activating proinflammatory responses.

A number of cell-surface molecules have been reported to act as putative receptors for Abeta peptides, among which the G protein-coupled formyl peptide receptor 2 (FPR2/ALX), this make the receptor an interesting target for Alzheimer's disease.

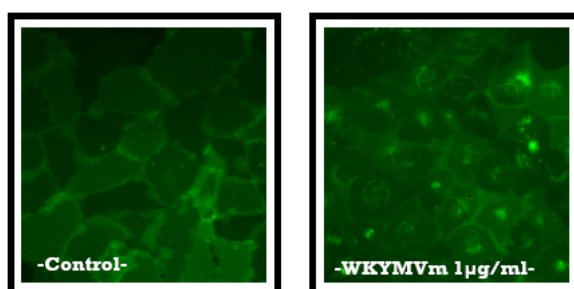
## Assay Characterization

Our expression plasmid containing the coding sequence of human Formyl peptide receptor 2 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 **FPR2/ALX** RT-PCR.

## Activation and Internalization assay for FPR2/ALX-tGFP ( $EC_{50} = 1.5 \times 10^{-5}$ $\mu\text{g/ml}$ )

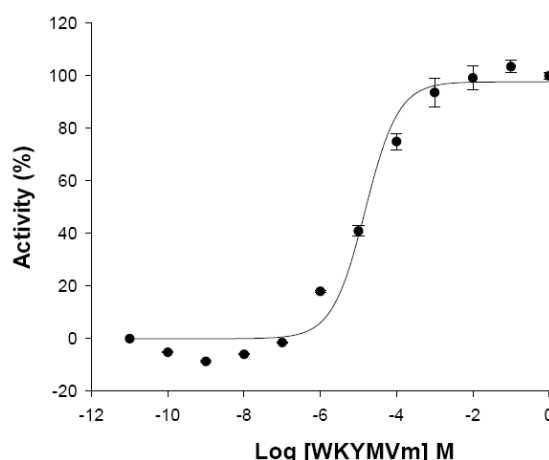


**Fig2.** Internalization of **FPR2/ALX** stimulated with **WKYMVm**. Concentrations from 0 to 1  $\mu\text{g/ml}$  were tested for 3h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Bioimager from BD Biosciences.

## Assay Details

U2OS cells, stably expressing human Formyl peptide receptor 2 tagged in the N-terminus with tGFP protein, were stimulated with increasing concentrations of **human WKYMVm** during 3 h. After the treatment an accumulation of fluorescence was observed around nucleus. Nuclei were stained with DAPI.

FPR2 fluorescence redistribution was determined measuring the fluorescence granularity surrounding the nuclei using image analysis algorithms.



**Fig3.** Concentration response curve for **WKYMVm** in Formyl peptide receptor 2 cell line.

Cells were treated with 12 log dilution series ( $n=6$ ). The  $EC_{50}$  for **WKYMVm** was  $1.5 \times 10^{-5}$   $\mu\text{g/ml}$  after a treatment of 3 h with the agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (1  $\mu\text{g/ml}$ ). The internalization assay was validated with an average of  $Z' = 0.7 \pm 0.02$  for High Content Screening.