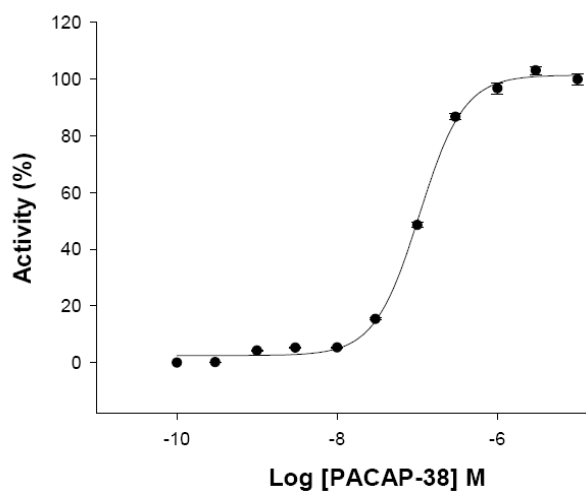
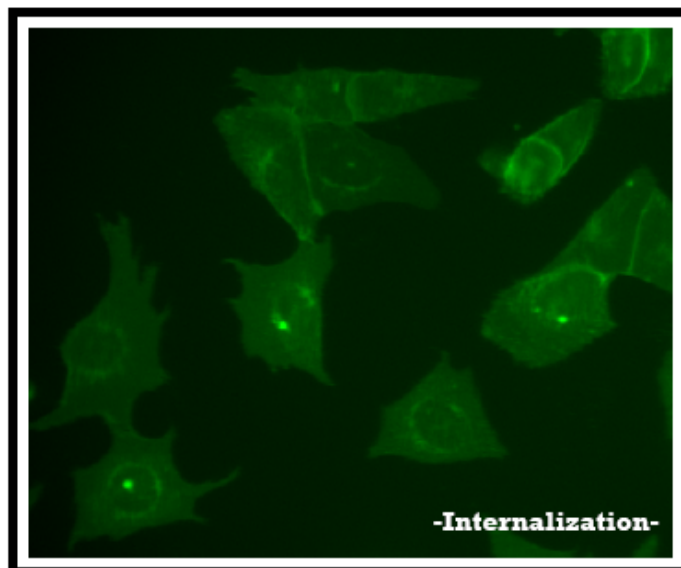
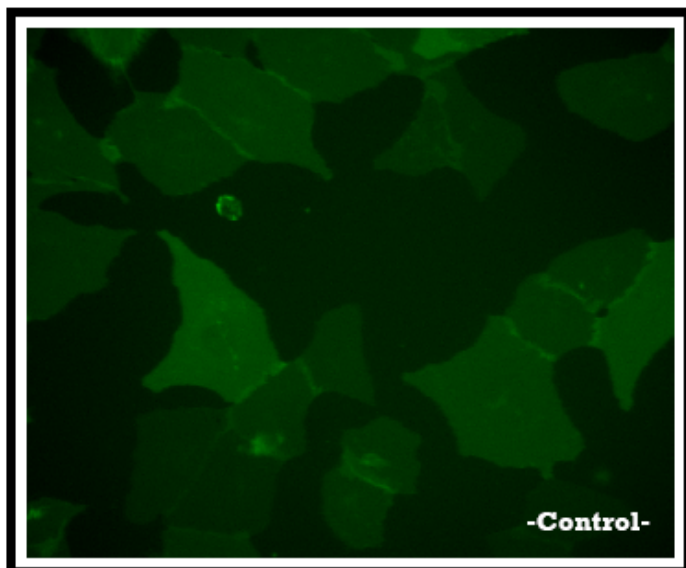


## RECEPTOR INTERNALIZATION ASSAYS

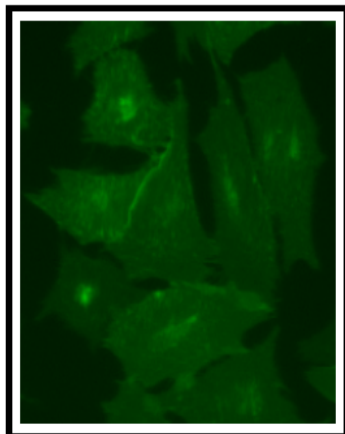
- PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE TYPE I RECEPTOR -



**Product name:** ADCYAP1R1-tGFP (PAC1-tGFP) / U2OS cell line

**EC<sub>50</sub> PACAP-38:**  $1.06 \times 10^{-7}$  M

**Z':** 0.73+/- 0.02



**Product Name:** ADCYAP1R1-tGFP\_U2OS


**Reference:** P30214


**Rep. Official Full Name:** Pituitary adenylate cyclase-activating polypeptide type I receptor

**DNA Accession Number:** Gene Bank AY366498

**Host Cell:** U2OS

**References:**

 **P30214:** 2 vials of  $3 \times 10^6$  proliferative cells

 **P30214-DA:** 1 vial of  $2 \times 10^6$  division-arrested cells

**Storage:** Liquid Nitrogen

### **Assay Briefly description**

Each vial of ADCYAP1R1 Internalization Assay Cell Line contains U2OS cells stably expressing human Pituitary adenylate cyclase-activating polypeptide type I receptor tagged in the N-terminus with tGFP protein.

Innoprot's ADCYAP1R1-tGFP Internalization Assay Cell Line has been designed to assay potential agonists/ antagonists against ADCYAP1R1, 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 **PACAP-38** as a ADCYAP1R1 agonist in a High Content Analysis (HCA).

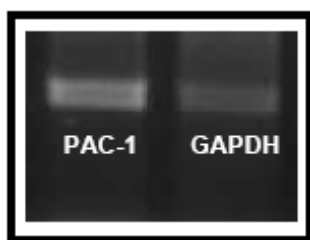
### **About ADCYAP1R1**

Pituitary adenylate cyclase-activating polypeptide type I receptor, also known as PAC1 is a protein that in humans is encoded by the ADCYAP1R1 gene. ADCYAP1R1 is a membrane-associated protein and shares significant homology with members of the glucagon/secretin receptor family. This receptor binds pituitary adenylate cyclase activating peptide (PACAP) mediating several biological activities and it is positively coupled to adenylate cyclase.

ADCYAP1R1 is expressed in the adrenal medulla, pancreatic acini, uterus, myenteric plexus and brain.

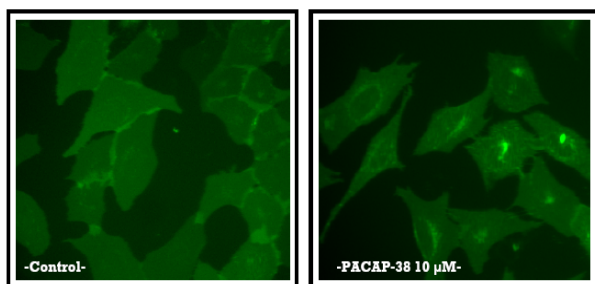
### Assay Characterization

Our expression plasmid containing the coding sequence of human Pituitary adenylate cyclase-activating polypeptide type I 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.** ADCYAP1R1 and GAPDH housekeeping gene RT-PCR.

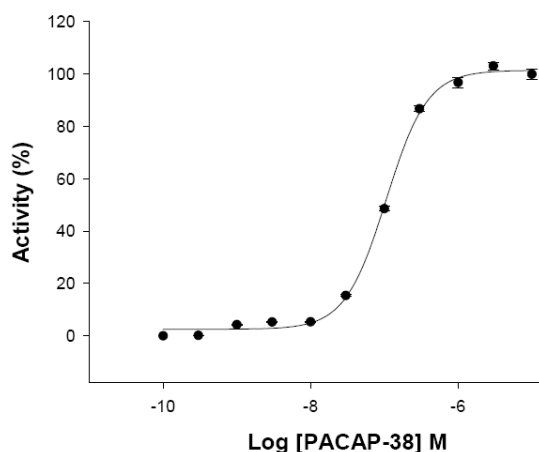
### **Activation and Internalization assay for ADCYAP1R1-tGFP (Ec50 = 1.06 x 10<sup>-7</sup> M)**



**Fig2.** Internalization of ADCYAP1R1 stimulated with PACAP-38. Concentrations from 0 to 10μM were tested for 24h. Activation and internalization processes were detected and analyzed using "BD Pathway 855" High-Content Biomager from BD Biosciences.

### Assay Details

U2OS cells, stably expressing human Pituitary adenylate cyclase-activating polypeptide type I receptor tagged in the N-terminus with tGFP protein, were stimulated with increasing concentrations of **human PACAP-38 during 24 h**. After the treatment an accumulation of fluorescence was observed around nucleus. Nuclei were stained with DAPI and fluorescence redistribution was determined measuring the increase of fluorescence surrounding the nuclei using image analysis algorithms.



**Fig3.** Concentration response curve for PACAP-38 in ADCYAP1R1 cell line. Cells were treated with 11 log dilution series (n=5). The Ec50 for PACAP-38 was ~ 1.06 x 10<sup>-7</sup> M after a treatment of 24h with the agonist. Cells were fixed and the nuclei were stained with DAPI. % Activity was calculated relative to positive (10μM). The internalization assay was validated with an average of Z' = 0.73 +/- 0.02 for High Content Screening.