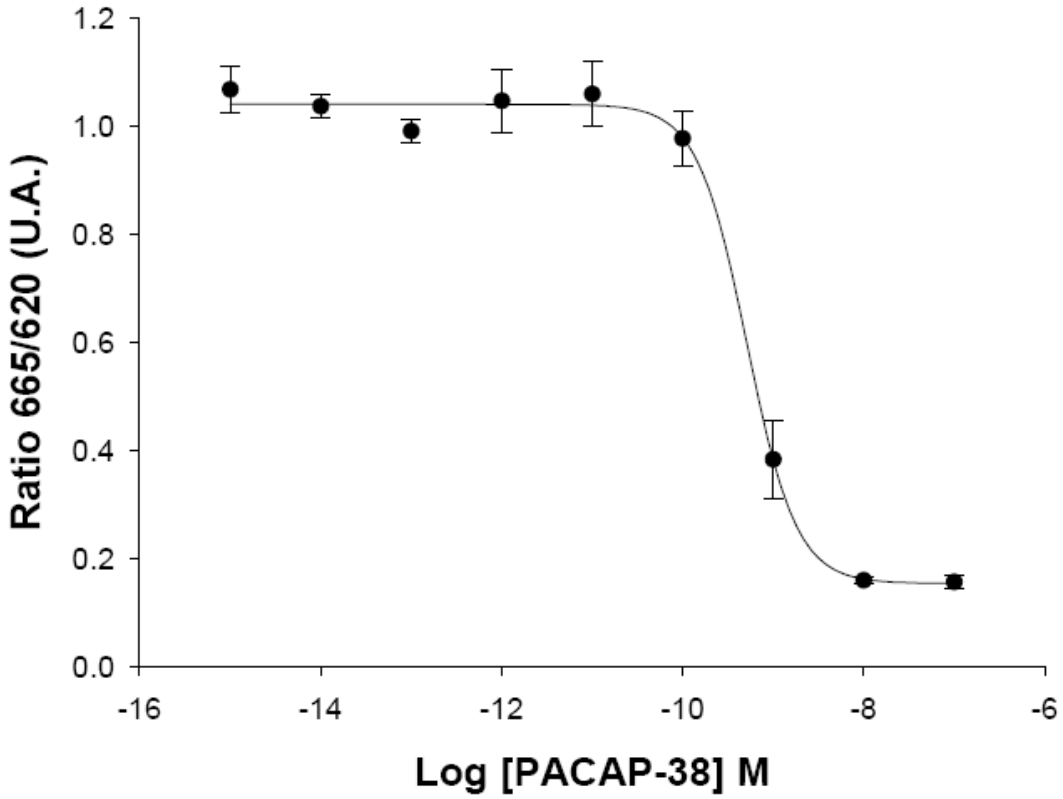
REF: P30114

HiTSeeker CELL LINES (LABEL-FREE GPCRS)

- PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE TYPE I RECEPTOR -



Product name: HiTSeeker ADCYAP1R1 (PACAPRI)

Ec_{50} PACAP-38: 5.13 x 10^{-10} M

Z´: 0.81+/- 0.01

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HiTSeeker CELL LINES (LABEL-FREE GPCRS)

PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE TYPE I RECEPTOR

Product Name:	ADCYAP1R1 (PACAPRI)/HEK293
Official Full Name:	Pituitary adenylate cyclase-activating polypeptide type I receptor
DNA Accesion Number:	GenBank: AY366498
Host Cell:	HEK293
Format:	Cryopreserved vials
Resistance:	G418 (Geneticin)
Size:	*P30114*: 2 vials of 3 x 10^6 proliferative cells *P30114-DA*: 1 vial of 2.5x10^6 division-arrested cells
Storage:	Liquid Nitrogen

Assay Briefly description

Each vial of HiTSeeker ADCYAP1R1 contains HEK293 cells stably expressing human Pituitary adenylate cyclase-activating polypeptide type I receptor (ADCYAP1R1) with no tag.

HiTSeeker ADCYAP1R1 cell line has been designed to assay compounds or analyze their capability to modulate Pituitary adenylate cyclase-activating polypeptide type I receptor. When the agonist binds to ADCYAP1R1 a G protein is activated, which in turn, triggers a cellular response mediated by second messengers (cAMP).

This cell line has been validated measuring cAMP increase in the cytosol. The high reproducibility of this assay allows monitoring ADCYAP1R1 activation process in High Throughput Screening.

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.

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Assay Characterization

Our expression plasmid contains the coding sequence of human ADCYAP1R1 receptor protein. Our plasmid was transfected in HEK293 cells. Resistant clones were obtained by limit dilution and receptor gene expression was tested by RT-PCR using GAPDH as internal control (Fig.1).

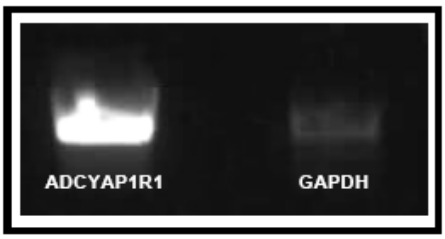

Fig.1. ADCYAP1R1 and GAPDH housekeeping gene RT-PCR.

The specific signal is inversely proportional to the concentration of native cAMP produced by the binding of the agonist to its receptor.
Fluorescence detection was recorded in a Multi-Mode Microplate Reader Synergy 2 from Biotek.

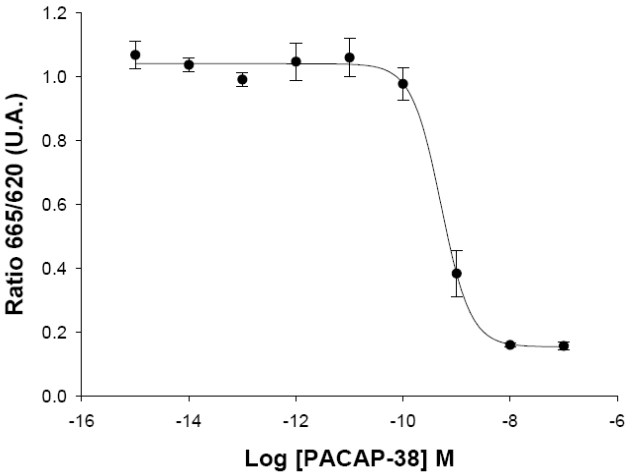

Fig.2. PACAP-38 dose response curve in cAMP assay. Cells were treated with PACAP-38. Concentrations from 0 to 10^{-7} M were tested by quadruplicate. The Ec50 for the PACAP-38 is ~ $5.13x10^{-10}$M. The cAMP assay was validated with a Z´=0.81 in the Multi-Mode Microplate Reader Synergy 2 from Biotek..

Validation of ADCYAP1R1 cell line

cAMP production assay (EC_{50}= $5.13x10^{-10}$M)

cAMP production was assessed using cAMP dynamic 2 kit (Cisbio). This kit contains labelled cAMP (620 nm) and an anti-cAMP antibody (665nm). Between these molecules occurs a fluorescence transfer (FRET). Native cAMP produced by cells (due to the binding of an agonist to its specific receptor) competes with the labelled cAMP producing a decrease of FRET detected by HTRF technology.

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